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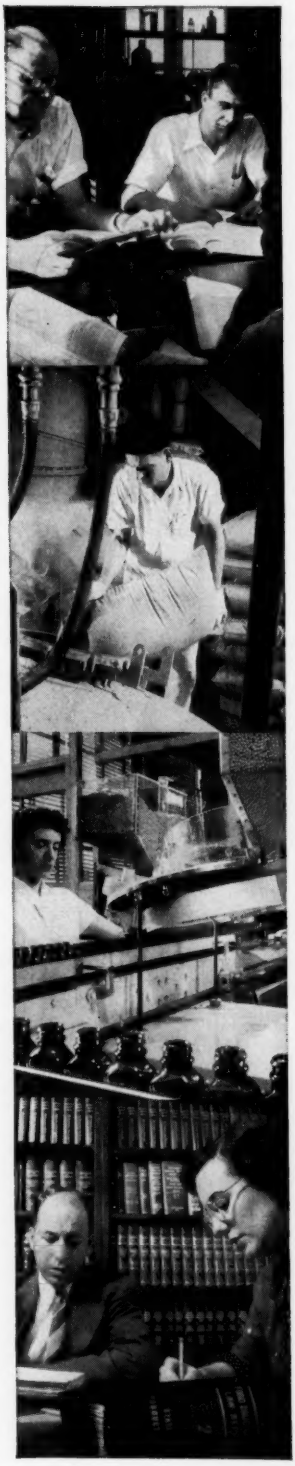

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THE POSSIBLE RELATIONSHIP OF ETIOCHOLANOLONE TO PERIODIC FEVER¶

INTRODUCTION

Periodic fever has been an enigma to clinicians since the original description by Hobart Reimann.[28] A patient with a history of periodic fever and an abnormality of steroid metabolism led us to investigate the 17-ketosteroid fractions in plasma and urine. This paper is a report of the relationship between symptoms of periodic disease and an apparently specific abnormality of etiocholanolone metabolism.

MATERIALS AND METHODS

Case Histories:

Case 1: W.S. was a 34-year-old physician with a 26-year history of periodic fever. The febrile episodes were associated with chills, lethargy, anorexia, malaise, depression, headache, muscle pain, and weight loss. The temperature during attacks ranged from 100° to 106° and was usually accompanied by a leukocytosis.

The first attack had occurred at the age of 8 and lasted approximately 6 months. At the age of 16 he had his most severe attack and was acutely ill with all of the above symptoms plus hypotension. At this time he was given adrenal cortical extract with dramatic improvement in his symptoms and sense of well-being. During the ensuing years he had numerous attacks lasting from 2 to 24 weeks. Investigative procedures for the diagnosis of fever of undetermined origin had always been negative, and antibiotics produced no change in the clinical picture except on those occasions when a specific infection was present.

In December, 1956, he was seen with complaints of malaise, anorexia, marked fatigue, and salt craving. He was afebrile at this time. Studies of adrenocortical function at the University of Virginia Hospital revealed urinary 17-ketosteroids rang-

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ing from 23 to 40 mg. per 24 hours; a fasting plasma 17-hydroxycorticoid level of 20 micrograms per 100 ml. (normal 4-30 micrograms) with no response to 25 mg. of ACTH intravenously over a 4-hour period on two separate occasions; and a urinary pregnanediol of 1.57 mg. per 24 hours (normal 0.3 mg.). The serum electrolytes were normal at this time. The urinary 17-ketosteroids were partially suppressed by the administration of 2 mg. of 9-α-fluorohydrocortisone daily for 10 days. In March, 1957, because of his symptomatology and the above laboratory findings he was treated with 37.5 mg. daily of cortisone with definite improvement in his symptoms and sense of well-being. The tentative diagnosis was adreno-genital syndrome although there was no history of precocious sexual development. He had noted severe acne since puberty and body hair was thought to be excessive.

In November, 1957, he had the beginning of another episode of fever which was still present when he was transferred from the University of Virginia Hospital to the Yale-New Haven Medical Center in January, 1958. During this attack his fever had reached 104° F. in spite of as much as 125 mg. of cortisone acetate daily.

The patient appeared healthy except for a pulse of 120 and a moderate fever. The liver was palpable 4 centimeters below the right costal margin and a spleen tip could be felt. According to the history these findings had been present since 1932. Firm, discrete 0.5-1.0 cm. cervical and axillary lymph nodes were also palpable. During his hospitalization the patient had temperature spikes of 2 to 5 days' duration not relieved by aspirin, but relieved by cortisone. When ACTH was administered, there was an exacerbation of the symptoms.

Laboratory studies revealed a leukocytosis of 20 to 30 thousand with a shift to the left, and hemoglobin values of 10 to 12 grams per cent. Serum total protein was 6.37 gm. per cent with 2.57 gm. of albumin and 3.80 gm. of globulin per 100 cc. Numerous other laboratory studies including multiple cultures, serologic tests, and searches for parasites, and lupus erythematosus also were negative. Liver and lymph node biopsies were normal.

Steroid studies revealed normal urinary pregnanetriol excretion[9] (1.6 mg. per 24 hours). The urinary 17-ketosteroids before ACTH were 6.7 mg. and afterwards rose to 11.1 and 12.8 mg., according to a modified method of Holtorff and Koch.[9] Ketogenic steroids before ACTH were excreted at a rate of 15.8 mg. per day and remained at this level after ACTH.[10]

Case 2: M.K., a 44-year-old man, had had repeated attacks every 3 to 4 weeks which lasted 3 to 4 days. These episodes consisted of vague epigastric distress with radiation to the right upper quadrant, fever as high as 103° F., anorexia, and a cough productive of "cheesylike" sputum. Bronchograms and sputum examinations in the past have always been normal. During one attack, adrenocorticotropin exacerbated the symptoms, but either prednisone or cortisone relieved them. The patient's father and brother had similar symptoms which were never completely investigated.

Physical examination revealed a thin, very apprehensive man complaining of pain in the right upper quadrant with tenderness to palpation. Blood pressure was 110 systolic and 70 diastolic. Physical examination was otherwise entirely within normal limits.

The white blood count was 13,600 with a shift to the left with normal eosinophile counts. Serum total proteins were 7.45 gm. per cent with 3.57 gm. of albumin and 3.88 gm. of globulin per 100 cc. Repeated cultures, skin tests, and agglutinations for a wide variety of infectious agents were unrevealing.

METHODS

Plasma:

Blood was obtained from the first patient during the febrile state, and a second sample was studied 8 weeks later while he was afebrile and receiving 25 mg. of cortisol daily. A single sample was obtained from the second patient while he was febrile.

About 50 ml. of plasma was obtained in each instance. The plasma proteins were precipitated with absolute alcohol and centrifuged.[5] The protein precipitate was extracted three times with 60 ml. of *n*-butanol; the alcohol and butanol extracts were pooled and dried in a rotating vacuum evaporator. The extract was dissolved in 70 per cent methanol and partitioned three times with hexane. The methanol phase was then concentrated *in vacuo* to a volume of about 1.0 ml. and streaked onto Whatman No. 3MM paper, 47 centimeters in length and 10 centimeters wide for electrophoresis.

Electrophoresis was performed in 0.1 molar barbital buffer with 20 per cent alcohol by volume at pH 8.6 at 400 volts and 20 m.a. for four hours. Twenty microgram standards of tetrahydrocortisone glucuronide and androsterone sulfate were spotted adjacent to the streaked extract. The glucuronide standard moved 8.5 centimeters toward the anode and the sulfate moved 11.0 centimeters toward the anode. The sulfate bands were located by cutting off the strip and dipping into Zimmermann reagent.[14] Glucuronide band was located by dipping the strip into triphenyltetrazolium chloride reagent.[13] The glucuronide and sulfate bands were cut out from the paper and eluted with 70 per cent methanol at 36° C. with shaking for two hours. Nonconjugated steroids which remained at the origin were cut out and eluted with methanol: chloroform (1:1) for two hours at 36°.

Paper chromatography was performed using washed Whatman No. 1 paper which was cut into strips 4 cm. wide and 50 cm. long. Beakers were placed beneath the strips to collect the effluent flows. The nonconjugated steroids were initially chromatographed in a Bush B5 system, 50 per cent methanol: benzene[8] to separate reduced corticosteroids from the 19-carbon steroids. The latter, being less polar than the corticosteroids, were collected in a beaker and re-chromatographed in System I., benzene:Skellysolve C®:methanol:water (Bush B-3), 33:66:80:20[9], along with reference standards of androsterone, dehydroisoandrosterone, and etiocholanolone. The C-19 steroids were also identified by their mobility in a heptane: propylene glycol system.[10] The glucuronide eluate was chromatographed in *n*-butyl acetate, *n*-butanol, 10 per cent acetic acid (80:20:100),[15] along with reference standards of tetrahydrocortisone glucuronide and tetrahydrocortisol glucuronide. The sulfate eluates from the electrophoresis were chromatographed in System 2, *n*-butyl acetate: *n*-butanol: 10 per cent formic acid (80:20:100),[16] with reference standards of etiocholanolone sulfate, dehydroisoandrosterone sulfate, and androsterone sulfate. The paper chromatograms were equilibrated for 12 to 16 hours and descending chromatography performed for 6 hours at 25 27° C.*

The C-19 steroids were identified by their mobility characteristics after staining with Zimmermann reagent. The glucuronides separated by paper chromatography were measured by a micro-carbazole test in a Beckman DU spectrophotometer at a wave length of 530 mμ.[5]

After the C-19 steroid obtained from patient M.K. was purified in the chromatographic systems 1 and 3 (Fig. 2), sublimation *in vacuo* was performed. The melting

* The heptane-propylene glycol system was run for 24 hours.

point of the crystalline material thus obtained was measured in a Fisher melting point apparatus.

Urine:

A 24-hour urine was collected from patient W.S. in the Yale-New Haven Medical Center when the patient was not on steroids, although he was having an attack of periodic fever. A second 24-hour urine was collected while the patient was afebrile, 8 weeks later. The urines were hydrolyzed with beef liver beta-glucuronidase, 300 units per ml. (Ketodase®*) at pH 4.5 (sodium acetate buffer) and 37° C. for 72 hours. At the end of the incubation period the urine was extracted continuously for 48 hours at pH 1 (sulfuric acid) with ether. The ether was washed with a solution of 5 per cent sodium hydroxide until pigment-free and then with water until neutral. The washings were added to the urine after having been neutralized with sulfuric acid. The urine was then boiled for 60 minutes and extracted three times manually with equal volumes of ether. The ether phase was washed as described above, combined with the first extract, and dried *in vacuo*. Traces of water were removed with benzene. The crude extract obtained in this fashion was chromatographed on an alumina column in the gradient elution system described by Lakshmanan and Lieberman[12] using 700 ml. of redistilled benzene and a gradient of 200 ml. of 4 per cent ethanol and benzene. The eluate was collected in 100 fractions. Zimmermann reactions (Holtorff and Koch) were carried out on 1 ml. aliquots from each fraction. The tubes corresponding to the peaks of Zimmermann material obtained in this fashion were then pooled and dried *in vacuo*. Identification by infrared spectrophotometry was performed in a Perkin-Elmer double beam spectrophotometer Model 21. The peaks containing androsterone and etiocholanolone were further analyzed for the presence of the delta-9 analogues according to the method of Bitman *et al.*[2]

RESULTS

Plasma:

There was less than 0.3 ug./100 ml. of the glucuronides of tetrahydrocortisone and tetrahydrocortisol in the plasma of W.S. during the febrile phase. The level of the total 17-ketosteroid sulfate was less than 2.0 ug. in the febrile plasma. Only one 17-ketosteroid was present, i.e., nonconjugated etiocholanolone as detected on chromatogram I (Fig. 1). The sulfate region from the afebrile plasma of patient W.S., when chromotographed in System 2 (chromatogram II, Fig. 1), revealed three Zimmermann-reacting areas. The first was an unidentified polar compound. The other compounds had mobilities identical with those of the pure reference compounds, dehydroisoandrosterone sulfate (marked D) and androsterone sulfate (marked A). No unconjugated 17-ketosteroids were detectable in the plasma drawn during the afebrile phase.

There were no detectable 17-ketosteroid sulfates or glucuronides present in the plasma of M.K. while he was febrile. On paper chromatography,

* Ketodase®—Warner—Chillcott Co.

etiocholanolone was recovered from the nonconjugated steroid area of the electrophoretogram and identified in Systems 1 and 3 (chromatograms III and V, Fig. 2). The crystalline material obtained by sublimation from the area corresponding to etiocholanolone on chromatogram V (Fig. 2) revealed a melting point of 149 to 150° C. The sulfuric acid spectrum of this substance in a Cary recording spectrophotometer revealed one peak at 312 mμ. This evidence conclusively identifies the compound as etiocholanolone. It is of interest to note that free (nonpyrogenic[31]) androsterone was also detected.

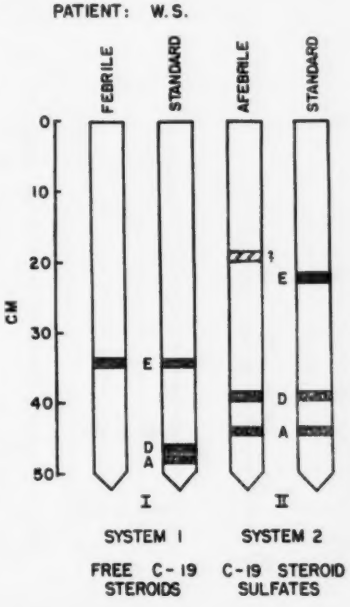

FIG. 1. The paper chromatography of plasma 17-ketosteroids. Roman numerals refer to the number of the chromatogram. The arabic numbers refer to the chromatography system employed.

E = Etiocholanolone
D = Dehydroisoandrosterone
A = Androsterone

System 1—benzene: Skellysolve C®; methanol; H_2O (33:66, 80:20)

System 2—butyl acetate; *n*-butanol; 10 per cent formic acid (80:20:100)

Urine:

In the urine of W.S. obtained during the afebrile phase there were four major peaks: androsterone-2.36 mg., etiocholanolone-3.97 mg., 11-oxy 17-ketosteroids plus △9-11-desoxy-17 ketosteroids-10.5 mg. Androsterone and etiocholanolone appeared in their characteristic position in the chromatography (Fig. 3), and were identified by their spectra in H_2SO_4 and infrared spectrophotometry (Fig. 4). The febrile urine of W.S. revealed one main peak consisting of etiocholanolone (3.2 mg. per 24 hours). A more polar compound (11-oxy-17-ketosteroid of undetermined nature) was also eluted. Figure 4 shows the fingerprint region of the infrared spectrum of the steroid obtained from the urine of W.S. during the febrile period. The identity of this infrared spectrum with that of etiocholanolone may be seen by comparison with the superimposed tracing of a pure standard of etiocholanolone. There were no 3-β-hydroxy 17-ketosteroids present in either the febrile or afebrile urine of patient W.S.

DISCUSSION

In both of the patients free (i.e., nonconjugated) etiocholanolone was demonstrated in the plasma at the time of the symptoms of periodic fever. In W.S., no free etiocholanolone was found during a period when symp-

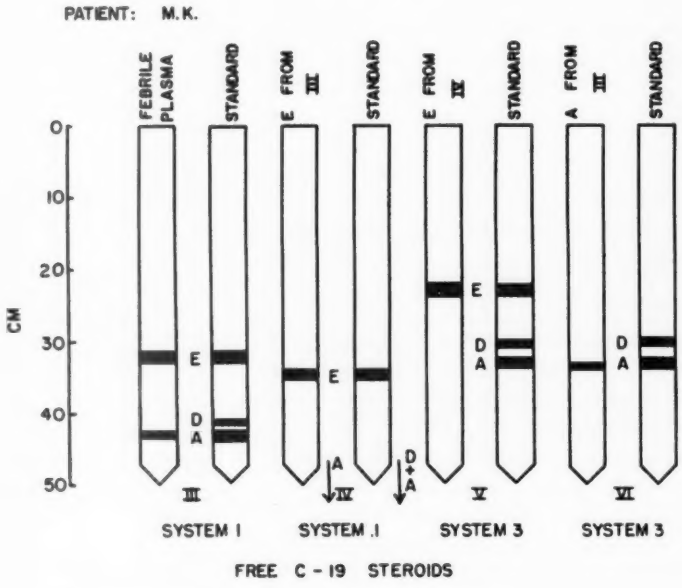

FIG. 2. Paper chromatography of plasma 17-ketosteroids.
E = Etiocholanolone
D = Dehydroisoandrosterone
A = Androsterone
System 1—benzene: Skellysolve C®; methanol, H_2O (33:66:80:20)
System 3—heptane, propylene glycol

toms were absent. A somewhat similar situation has been reported in a 7-year-old boy with the adrenogenital syndrome by Gonzales and Gardner,[7] who found elevated total plasma ketosteroids during peculiar periods of paroxysmal fever, flushing, and pain in the abdomen and head, chilly sensations, prostration, and vomiting.

The demonstration of nonconjugated etiocholanolone appears particularly significant in our patients since in normal individuals 17-ketosteroids

are present in the plasma only in conjugated form.* It is therefore reasonable to suggest that the presence of unconjugated etiocholanolone in the plasma of these patients may be associated in some way with their episodes of fever, malaise, headache, nausea, myalgia, and prostration.

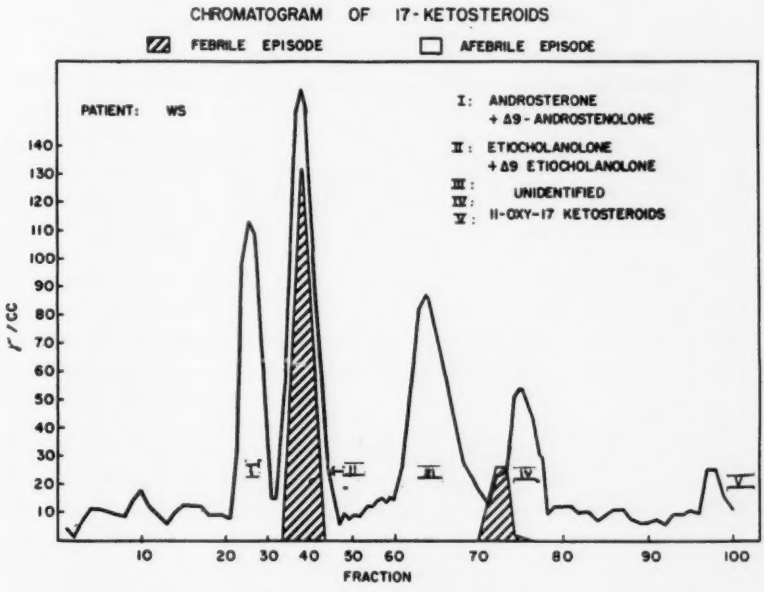

FIG. 3. Fractionation of 24-hour urinary 17-ketosteroids on alumina column. The shaded areas represent the quantity of 17-ketosteroids obtained from patient W.S. during the febrile period. The open areas represent the amount of individual 17-ketosteroids present during an afebrile period.

Kappas, Hellman, Fukushima, and Gallagher[11] reported that the intramuscular injection of etiocholanolone to normal human volunteers produced shaking chills followed by muscular aches and pains, anorexia, sweating, headache, and fever as high as 103° F. Leukocytosis with a shift to the left of the differential count also occurred. When smaller doses of

* Dubrulle and Claeys[6] measured unconjugated 17-ketosteroids in plasma and found 7-28 ug/100 ml. of plasma in 16 normal adolescents between the ages of 14 and 17. This finding is contrary to our own observations and those reported by Clayton, Bongiovanni, and Papadatos[4] and Migeon and Plager[15] who found that the amount of unconjugated steroid in plasma to be insignificant or absent.

etiocholanolone were injected daily, the fever continued for the duration of the injections without the production of tolerance. Cortisone pretreatment reduced the febrile reaction. The pyrogenic effect of etiocholanolone was unique, since androsterone (3α-hydroxyandrostane-17-one), epian-

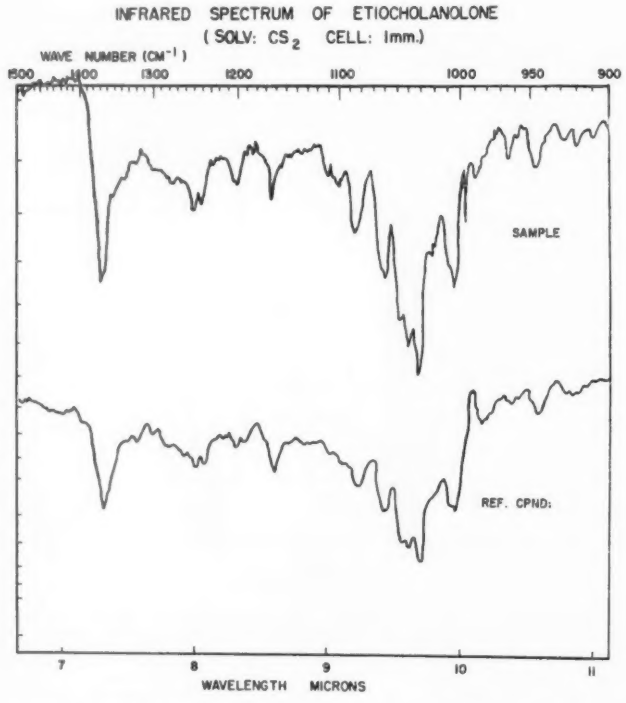

FIG. 4. Patient W.S.: Finger-print region of infrared spectrum of etiocholanolone, the *sample* refers to the material isolated from the urine during febrile period (Shaded area, Fig. 3). *Ref. Cpnd* is the reference compound of pure etiocholanolone.

drosterone (3β-hydroxyandrostane-17-one), and 3β-hydroxyetiocholane-17-one did not produce fever. Segaloff, Bowers, Gordon, Schlosser, and Murison also report the occurrence of untoward reactions and hyperpyrexia in 15 of 18 patients with advanced breast carcinoma who received intramuscular etiocholanolone.[20] It therefore seems likely that the symptoms of this disease may be produced by the release into the blood stream of endogenous unconjugated etiocholanolone.

This hypothesis is further supported by the observation that adrenocorticotropin aggravates the symptoms while corticosteroids such as cortisone or prednisone ameliorate them.

The fact that W.S. had a reduced ability to form 17-hydroxycorticoids and a tendency to excrete increased quantities of urinary 17-ketosteroids, at least at one time in his course, and that the symptoms were benefitted by cortisone treatment suggests that he may have a block in the synthesis of cortisol analogous to that seen in true adrenogenital patients such as have been described by Wilkins.[21] One might postulate that in this type of patient failure to hydroxylate the 21-carbon results ultimately in an overproduction of Δ4-androstene, 3, 17-dione which is reduced to etiocholanolone elsewhere in the body (presumably in the liver). This hypothesis seems unlikely for two reasons: first because in the ordinary adrenogenital patient no defect in 11-β-hydroxylation occurs,[10] so that much of the excess of 17-ketosteroid is hydroxylated on the 11 carbon; this was not the case in our patients. Moreover, overproduction of etiocholanolone is not, in itself, associated with fever, since the conjugated form of the steroid may be excreted in large quantities without associated symptoms. It appears that a failure to conjugate the substance must also be present in order for symptoms to occur.

A more likely hypothesis, therefore, points to the liver as a possible source of the difficulty. The reduction of androgens normally results in the production of androsterone and etiocholanolone, which are promptly conjugated. Our patients may suffer from an episodic inability to conjugate the steroids, associated with an increased production of etiocholanolone in preference to androsterone as an end product of androgen reduction.

There is no definite evidence of hepatic disease in these patients, but the hepatic and splenic enlargement in W.S., associated with abnormal plasma protein patterns in both patients, suggests that other parameters of hepatic function may be disturbed.

Although etiocholanolone causes fever in human beings, it is not like bacterial "pyrogen" since no tolerance develops to its administration.[1, 11] Moreover, etiocholanolone does not cause fever in rabbits.[17] For these reasons it seems unlikely that etiocholanolone plays an important part in the development of ordinary fever in human beings.

SUMMARY

Unconjugated etiocholanolone has been demonstrated in the plasma of two patients suffering from periodic attacks of fever, malaise, nausea, and myalgia (periodic disease). The steroid was not present in the unconjugated form between attacks in one of the subjects. The only 17-keto-

steroid present in measurable quantities in the urine of one patient during an attack was etiocholanolone, whereas between attacks a normal distribution of urinary 17-ketosteroids was present. The nature of the etiocholanolone was proven by chromatographic mobility characteristics, melting point, sulfuric acid spectrum (in plasma), and these plus infrared spectrum (in urine). It is proposed that the attacks of periodic fever in these patients are a result of the presence of unconjugated etiocholanolone in the plasma. Possible mechanisms for this abnormality are discussed.

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EXPERIMENTAL PYELONEPHRITIS. IV. OBSERVATIONS ON INFECTIONS RESULTING FROM DIRECT INOCULATION OF BACTERIA IN DIFFERENT ZONES OF THE KIDNEY†

INTRODUCTION

The preceding report in this series[5] described an enhanced susceptibility to *E. coli* pyelonephritis which resulted from the presence of a small mechanical injury in the medulla of the kidney. The acute pyogenic infection which developed was restricted to the distribution of those nephrons with collecting tubules passing through the area of injury. The evidence appeared to suggest, therefore, that obstruction of urine flow, with resultant increased intratubular pressure, is capable of altering susceptibility to infection. It was also observed that within the affected area multiplication of bacteria appeared to begin earlier in the medulla than in the cortex, suggesting a difference in capacity of these tissues to support the initiation of bacterial infection.

In the previous work the bacteria were inoculated either into the blood stream or the bladder urine. The present experiments were designed to study the effect of injecting bacteria directly into cortex or medulla.

MATERIALS AND METHODS

Except where specified, the materials and methods were the same as described in preceding reports from this laboratory.[3, 5] All experiments were carried out in rabbits.

Injection of bacteria into the kidney. The abdomen was entered through a midline incision. Because of easier accessibility, all injections were made into the left kidney. A 27-gauge, 2.0-cm. hypodermic needle and a syringe calibrated to 1/100 ml. were used to inject the bacterial suspension, in a volume of 0.05 ml. For medullary inoculations the needle was inserted perpendicular to the surface of the kidney 1.3 cm. medial to the lateral margin, midway between the upper and lower poles, and to a depth of 1.0-1.2 cm. The cortex was injected by passing the needle 1-2 mm. beneath the surface of the lateral margin of the kidney for a distance of 1.2 cm. Immediately after removal

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of the needle from the papilla, slight pressure was applied over the puncture site for one to two minutes. After cortical injections, pressure was applied between the site of puncture and the tip of the needle before withdrawing the needle. The possibility of leakage of fluid from the puncture holes will be considered subsequently.

Each animal received only one injection. Four days later the kidney was removed and subjected to gross examination. Normal-appearing kidneys were cultured whole to be certain that all infections would be detected. In the case of obstructed kidneys, where the areas of infection were obvious on gross examination, the kidneys were divided and one-half was used for histological study, the other for quantitative culture.

Ligation of the ureter. The left ureter was doubly ligated and then divided midway between kidney and bladder. Injection of bacteria into the kidney substance was made about 5 minutes later.

Osmotic diuresis. Ten ml. of 20 per cent dextrose in water were injected intravenously.

EXPERIMENTAL

Number of organisms necessary to initiate infection. Cortex. Serial tenfold dilutions of *E. coli* were injected into the cortex. It was found that a considerable number of organisms was necessary to produce infection, since bacteria were not detected in 12 kidneys injected with 10^3 or 10^4 organisms. On the other hand, when the inoculum was increased to 10^5 or 10^6 organisms, abscess formation with positive culture was found in 8 of 12 animals.

The abscesses produced by cortical inoculation varied in diameter from 1 to 5 mm. and developed only at the site of injection without apparent involvement of the medulla.

Medulla. In contrast to the relative resistance to infection in cortical tissue, the medulla was found to be highly susceptible. Fewer than 10 organisms were able to produce infection in this area in contrast to the 10^5 usually required in the cortex (Table 1).

The infection caused by medullary inoculation consisted of a collection of small abscesses in a wedge-shaped area, with the apex in the medulla and the base in the cortex. The nephrons involved seemed to be those tributary to the collecting tubules passing through the site of injection. Infection did not develop along the path of the needle. The area of involvement was sharply demarcated from the surrounding normal kidney tissue, without a tendency to spread laterally in either cortex or medulla, similar to that shown in Figure 1. Despite the profusion of bacteria and pus cells in the kidney pelvis, infection was detected only in areas affected by the original injury. The appearance of these lesions was similar to that which had been observed in the previous experiments,[5] by injuring an area in the medulla and then injecting *E. coli* intravenously.

Effect of the trauma of needle puncture on susceptibility to infection. Hypodermic needles were inserted into the left renal papilla of 4 rabbits, as for the inoculation of bacteria. In 2, the needle was promptly removed and in the remaining 2, 0.05 ml. sterile saline was injected. Microscopic examination 4 days later revealed the presence of intrarenal hydronephrosis in all 4 animals similar to the change evoked by cautery injury of the medulla.[5] In another group of 4 animals prepared similarly, 0.5 ml. of a 4-hour culture of *E. coli* was injected into an ear vein. Four days later all were found to have acute pyelonephritis similar to that obtained by injecting bacteria into the kidney (Fig. 1).

TABLE 1. INCIDENCE OF INFECTION RESULTING FROM INJECTION OF BACTERIA INTO CORTEX OR MEDULLA OF THE NORMAL KIDNEY

		Site of injection		
		Cortex	*Papilla*	*Colonies E. coli per kidney*
Number of organisms injected	<10		1/4	10^4
	10^1		4/7	10^3, 10^4, 10^5, 10^6
	10^2		3/4	10^5, 10^6, 10^7
	10^3	0/6	4/4	10^4, 10^5, 10^6, 10^8
	10^4	0/6		
	10^5	3/6		10^1, 10^2, 10^3
	10^6	5/6		10^3, 10^4, 10^5, 10^6, 10^8

Number of infections/number of tests made.

It is clear, then, that injection of small amounts of fluid or, indeed, the mere insertion of a hypodermic needle, into the medulla of the kidney resulted in the formation of an area of intrarenal hydronephrosis with its attendant susceptibility to infection.

This finding raised the possibility that the increased susceptibility of the medulla as compared with the cortex was due only to the fact that injury or local injection into the medulla necessarily caused some obstruction of urine flow. In contrast, cortical injection would not have the same capacity to block the flow of urine throughout a group of nephrons.

In order to determine whether cortical tissue is also highly susceptible to infection *in the presence of obstruction,* the experiments were repeated, and the inoculations were made into kidneys with ligated ureters, that is, with a static column of urine exerting increased pressure throughout the length of the nephrons.[3]

FIG. 1. The appearance of a kidney 4 days after papillary injection of 0.05 ml. sterile saline, followed immediately by the intravenous inoculation of 0.5 ml. of a 4-hour culture of *E. coli*. Note the limitation of the infection to the area of nephrons which drain into the site of the papillary injection. (The light streaks on the pole of the kidney opposite to that infected are photographic artefacts.)

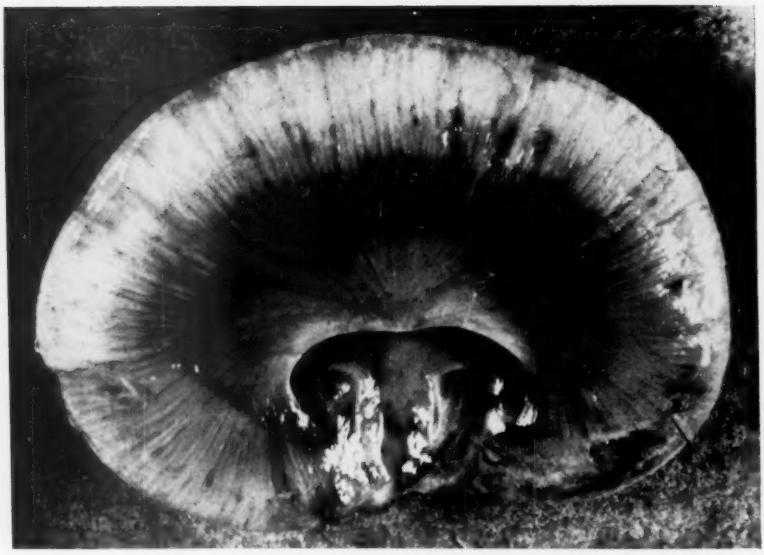

FIG. 2. A kidney 4 days following ureteral ligation and the intrarenal injection of organisms. The infection is oriented along the course of nephrons but involves the whole kidney. This type of infection resulted when sufficient bacteria were inoculated into either cortex or medulla of the obstructed kidney. This specimen was obtained following the injection of 10^3 organisms into the cortex of a kidney with its ureter ligated during glucose diuresis.

Injection of E. coli into kidneys with ligated ureters. The kidneys with ligated ureters were injected with *E. coli* in the same fashion as had been done in the preceding experiments. The results are shown in Table 2. It was found that the inocula required to infect the cortex and medulla did not differ greatly from those required for normal kidneys, since the lowest number of organisms establishing infection in the cortex was 10^4, whereas in the medulla fewer than 10 bacteria were again found to be capable of establishing infection.

It is of interest to note that, in contrast to the infection in unobstructed kidneys, the end result in these preparations was a diffuse process, in-

TABLE 2. INCIDENCE OF INFECTION RESULTING FROM INJECTION OF BACTERIA INTO CORTEX OR MEDULLA OF KIDNEY WITH OBSTRUCTED URETER

		Site of injection		
		Cortex	*Papilla*	*Colonies E. coli per kidney*
Number of organisms injected	<10		2/3	10^8, 10^9
	10^1		2/2	10^8, 10^9
	10^3	0/6		
	10^4	3/6		10^8, 10^9, 10^9
	10^6	4/4		10^8, 10^9, 10^9, 10^9

Number of infections/number of tests made.

volving the entire kidney substance (Fig. 2). This developed whether the organisms were placed in the cortex or the medulla. It appears, then, that in the presence of ureteral obstruction infection does not remain confined to a wedge-shaped area of medulla and cortex, or to a circumscribed cortical lesion, but is, instead, now able to spread rapidly and involve the whole organ.

Injection of E. coli into obstructed kidneys during osmotic diuresis. The possibility had to be considered that in the forementioned experiments a general increase in intratubular pressure might not have developed until many minutes after ligating the ureter. Since the fate of the injected bacteria might be decided soon after injection, it was decided to repeat the study, in animals which, in addition to having a ureter ligated, were subjected to an osmotic diuresis by intravenous administration of 20 per cent glucose solution. Within a minute after the injection of the glucose solution the proximal portion of the ligated ureter and the obstructed kidney had become distended. It seems reasonable to assume, then, that an in-

creased pressure existed throughout the nephrons. The results obtained under these circumstances are given in Table 3. Despite addition of glucose to the urine and extracellular fluid, a difference in susceptibility of cortex and medulla was still present. Only 4 of 18 animals receiving cortical injections of 10^8 to 10^5 bacteria became infected, whereas infection was induced in 4 of 5 animals receiving smaller inoculations in the medulla. One of the results listed as positive following the cortical inoculation of 10^8 *E. coli* was unique in showing no gross evidence of abscess formation and was the only instance of its kind encountered. Microscopic study showed that the infection was limited to a few tiny subcapsular abscesses. Other-

TABLE 3. INCIDENCE OF INFECTION RESULTING FROM INJECTION OF BACTERIA INTO CORTEX OR MEDULLA OF KIDNEY WITH LIGATED URETER DURING OSMOTIC DIURESIS

		Site of injection		
		Cortex	*Papilla*	*Colonies E. coli per kidney*
Number of organisms injected	<10		0/1	
	10^1		4/4	10^9, 10^9, 10^9, 10^9
	10^6	2/9		$>10^9$, 10^9
	10^4	1/5		10^8
	10^5	1/4		10^8

Number of infections/number of tests made.

wise the lesions observed in these kidneys were similar to those described in obstructed kidneys not undergoing osmotic diuresis.

Consideration of factors bearing on the foregoing results. In searching for an explanation for the difference between the effects of cortical and medullary injection of bacteria, it was necessary to determine whether there were significant differences in the distribution of the organisms deposited in these areas.

1. *Placement.* One obvious possibility was that some of the medullary (papillary) injections were not placed properly and were either short of the mark or went through kidney parenchyma with resultant deposition of organisms in the renal pelvis. This may have taken place but would in fact tend to lower the incidence of infection after medullary injection in the normal kidney since, as has already been pointed out, normal unobstructed kidney tissue does not seem susceptible to infection by bacteria in the pelvis of the kidney.

2. *Leakage at the site of kidney puncture.* Escape of blood and bacteria from the puncture site after removal of the needle tended always to be more easily controlled after cortical injection than after medullary injection. In the kidney undergoing osmotic diuresis with a ligated ureter a small amount of leakage did occur but appeared to be comparable after injection into either site. It is not felt, therefore, that this factor influenced the results obtained.

TABLE 4. DISTRIBUTION OF E. COLI 5 MINUTES AFTER INJECTION INTO CORTEX OR PAPILLA

Size of inoculum (10^7 *E. coli*/.05 *ml.*)		*Urine* (*per ml.*)	*Blood* (*per ml.*)	*Kidney* (*whole organ*)
.05 ml.	Papilla	10^5	10^1	10^5
		10^6	10^2	—
		10^6	10^1	—
	Cortex	—	10^3	—
		0	10^2	—
		0	10^2	10^6
		0	10^3	10^5
		0	10^3	10^6
		0	10^3	10^5
		0	10^3	10^4
		0	10^3	10^5
.25 ml.	Cortex	0	—	—
		0	—	—
		0	—	—
1.0 ml.	Cortex	0	—	—
		0	—	—
		0	—	—

3. *Escape of bacteria into blood and urine following the injection.* Due to obvious structural differences between the renal cortex and medulla it was thought possible that significant loss of organism might occur because of variation in their accessibility to lymphatics, blood stream, and urine. To test this, samples of bladder urine, blood, and the whole kidney were cultured quantitatively five minutes after the inoculation of 10^7 organisms into cortex or medulla. It can be seen in Table 4 that the number of viable units recovered from the kidney after cortical or medullary injection is 10^1 to 10^3 lower than the number administered and that some of them had entered the blood stream. The numbers of organisms recovered from the blood stream and remaining in the kidney 5 minutes after injection were

approximately the same, whether the organisms were injected into the cortex or into the medulla. The urine cultures, however, showed a consistent difference, since organisms were always found in the urine after medullary, but never after cortical, injection. This was an unexpected finding and it was suggested therefore that a larger volume of inoculum might be more effective in causing bacteria to pass through nephrons. Cortical injections with five and twenty times the volume of inoculum used in the above studies were then tested. Even with these, no organisms were recovered from the urine 5 or 10 minutes after injection in 6 animals (Table 4). It is noteworthy that despite these observations, 2 of 5 urines obtained from animals with cortical abscesses resulting from injections into the cortex 4 days previously were found to contain 10^5 *E. coli* per ml. There was no evidence of infection other than at the point of inoculation well within the cortex. Thus, under these circumstances it would appear that material can be carried from cortex through the collecting system of tubules, into the urine.

DISCUSSION

Inoculating the medulla of the normal kidney with fewer than 10 bacterial cells was found to result in infection of the medulla which extended to the cortex. In contrast to this, approximately 100,000 organisms were required to produce infection when they were introduced into cortical tissue; furthermore, the process did not exhibit a tendency to spread down into the medulla. One might conclude from these observations that the normal medulla and cortex differ significantly in their susceptibility to infection. The mechanical procedure is similar for both injections, as is the attending tissue damage. However, this finding has to be interpreted in the light of the fact that medullary inoculation necessarily causes obstruction and intrarenal hydronephrosis, since injection of as little as 0.05 ml. of sterile saline into the renal medulla produced these effects. In fact, the mere passage of a #27-gauge needle into the medulla, without the injection of fluid, produced intrarenal hydronephrosis sufficient to cause infection following the intravenous inoculation of organisms.

In view of the obvious difference between the effects of the injections into cortex and medula, the observations were repeated in kidneys which were obstructed by ligating the ureter. It was thought that by thus obstructing the flow of urine in the ureter, the entire kidney would become hydronephrotic, i.e., there would be puddling of urine throughout the nephrons, resulting in an increase in intratubular pressure. It was anticipated that in such a preparation the difference between the effects of cortical and medullary injections might not be found. However, even in

the presence of ureteral obstruction it was still necessary to inject 10,000 or more organisms into the cortex in order to establish infection. Medullary tissue thus appears to be more susceptible to infection than cortical tissue *in the presence of obstruction.* The information is not at hand to make a conclusive statement regarding ability to infect normal medulla since it has not been possible to introduce bacteria directly into this area without in some way obstructing nephrons. The unobstructed medulla seems to be relatively resistant to infection since intravenous inoculation of bacteria in the normal animal results in the deposition of some bacteria in the renal medulla.[1,5] Further, despite the fact that some of the localized cortical abscesses resulted in the appearance of 10^5 organisms per ml. of bladder urine, medullary abscesses were not found. In addition, it was observed in a previous study that infecting the bladder urine of a rabbit with unilateral intrarenal hydronephrosis resulted in the infection of that kidney alone. The normal kidney was never found to be damaged.[5] It appears then that *E. coli* may be delivered to normal medullary tissue either by way of the blood stream, the nephron, or the ureter without causing infection.

The escape of bacteria into the pelvic urine following inoculation of the medulla is probably not the factor which determines the difference in susceptibility between medulla and cortex. The presence of large numbers of bacteria in the pelvic urine did not lead to infection of normal kidney tissue. It did, however, probably play a significant role in the subsequent course of infection in those kidneys with ligated ureters. As has been shown previously, injection of bacteria into the ureteral urine of an obstructed kidney is followed by rapid development of widespread renal infection.[4] We have found this to occur with the intraureteral injection of fewer than 10 organisms.

From the standpoint of the pathogenesis of pyelonephritis, the crucial issue is not the difference in susceptibility between obstructed cortex and medulla, but rather the explanation for the exquisite sensitivity of the medulla to infection when there is obstruction to urine flow. In considering this it is necessary to examine the consequences of hydronephrosis. The most obvious one is a static column of urine, which is capable of supporting multiplication of the test organism. The presence of this stagnant culture medium might be thought to provide the essential factor necessary for rapid bacterial growth. The experiments described here do not, however, support that hypothesis, since even under conditions of osmotic diuresis and ureteral ligation, the susceptibility of the cortex did not approach that of the medulla. Despite the fact that organisms injected in the normal cortex were not detected in bladder urine, it seems almost certain that rupture of urine-filled convoluted tubes would result from the injection procedure,

providing opportunity for local contact between bacteria and tubular contents. It is thought therefore that access to urine is not the deciding factor in the difference between the results of injections into cortex and medulla.

The roles played by the other effects of obstruction of urine flow are unknown. The increased pressure influences blood flow and lymphatic drainage and in addition undoubtedly brings about significant chemical alterations within the cells. Further studies are needed to determine whether any of these factors alters susceptibility to infection.

SUMMARY

Observations were made on the relative susceptibility of renal cortex and medulla to infection by inoculating colon bacilli directly into each zone.

Infection resulted from the introduction of fewer than 10 bacilli in the medulla, whereas 100,000 or more were usually required to establish infection in the cortex. The lesion which develops following cortical injection is an abscess localized to the site of inoculation. With medullary inoculation the area of infection is wedge-shaped, including both medulla and cortex, apparently involving all nephrons which drain through the site of medullary injury.

A factor which may play an important part in the apparent susceptibility of medullary tissue to infection is that the procedure of medullary puncture obstructs a group of collecting tubules, with the formation of a segment of intrarenal hydronephrosis. In the presence of obstruction of the entire kidney, however, there remains a difference between susceptibility of cortex and medulla to infection. Furthermore, when infection does become established in such a kidney, the end result is the same regardless of site of inoculation: a diffuse process involving the entire kidney substance.

The presence of large numbers of bacteria in the urine did not lead to infection of kidney substance except in the presence of obstruction.

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TELENCEPHALIC PROJECTIONS OF THE MIDLINE AND INTRALAMINAR NUCLEI IN THE CAT**

INTRODUCTION

Since 1942, when Morison and Dempsey[18] first published the finding that stimulation of certain subcortical structures in the region of the internal medullary lamina resulted in widely distributed "rhythmically recurrent cortical potentials," there has been a steadily increasing interest in the physiological and anatomical parameters of this so-called "recruiting response."

Subsequent investigations by Jasper[10] and by Starzl and Magoun[32] bore out, in general, the initial results of Morison and Dempsey but differed to a certain degree as to which cortical areas were affected and which subcortical structures, when stimulated, elicited the "recruiting response." The group of subcortical structures includes, according to most investigators, the midline and intralaminar nuclei, the centrum medianum, the anterior nucleus, and the anterior portions of the reticular complex of the thalamus. However, cortical activation has also been reported by some authors upon stimulation of the n. ventralis anterior,[32] n. medialis dorsalis,[1] n. reuniens,[10] n. parafascicularis,[30] n. postero-lateralis,[22] and the pulvinar.[32]

It has been postulated that the nuclei from which recruiting phenomenon can be elicited are the diencephalic components of a "central activating system"[13]† or "centrencephalic system"[30]‡ which would, by modulating all

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* Fourth-year medical student.

** The material here presented is taken from a thesis submitted to the Faculty of the School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine in 1958.

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† The "central activating system" is defined by Magoun as being composed of the brain stem reticular formation, tegmentum, subthalamus, dorsal hypothalamus, and the ventro-medial thalamus. All of these structures possess the common characteristic of producing cortical desynchronization upon stimulation.[13]

‡ The "centrencephalic system" is described by Penfield as "an area of the brain, separate from the motor and sensory cortex, in which the highest level of functional integration is to be found . . . a ganglionic area in which . . . those nervous mechanisms are to be found which are prerequisite to the existence of the intellectual activity and prerequisite to the initiation of the patterned stream of efferent impulses that produce the planned action of the conscious man. . . . The intralaminar systems of the thalamus and the reticular formation of the brain stem and the non-specific projection systems which have widespread connections with the cortex of both sides satisfy the definition [of a 'centrencephalic system']."[30]

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cortical activity, perhaps serve as the "neural substratum of consciousness" of Hughlings Jackson.[9]

The delineation of the neural pathways by which these structures so modify cortical activity, and thus behavior, is obviously an important one for an understanding of perceptual and learning processes and even mental illness. However, anatomical investigations of this region have lagged far behind the physiological studies.

Recent work has variously involved both basal ganglia and anterior rhinencephalon as possible routes of transmission of such diencephalic-mesencephalic influence on the isocortex. But very few studies have been designed to resolve the apparent discrepancies that have been reported. This study, therefore, was undertaken in an attempt to decide, by means of well-localized, superficial lesions of anterior rhinencephalic cortex, whether or not projections to this area from the midline and intralaminar nuclei do indeed exist.

MATERIALS AND METHODS

Surgical Procedures

Six cats were operated upon under Nembutal anesthesia. The left zygomatic arch and left temporal bone were removed, the dura opened and the brain retracted to reveal the left orbital surface. A sub-pial removal of prepyriform cortex was accomplished with a #19-gauge needle sucker. The dura was closed, the temporal muscle was sewn over the bony defect, and the skin closed in layers.

Anatomical Procedures

The animals were sacrificed 8 weeks after operation. Fixation was initiated by transcardial perfusion with saline, followed by 10 per cent formalin, and the brains subsequently stored in 10 per cent formalin for 1 week. They were then dehydrated in increasingly concentrated alcohol solutions, infused with 2 per cent, 5 per cent, and 10 per cent solutions of nitrocellulose and finally imbedded in 20 per cent nitrocellulose. Blocks were stored in 70 per cent alcohol until sectioned. Sections were cut of 50-micra thickness and were strained at 250-micra intervals, with the exception of those through the thalamus, which were stained at 100-micra intervals.

Tracings of sections at 1 mm. intervals were used in reconstructing and plotting the outlines of the orbital surface, the principal fissures and the lesion on graph paper. Tracings of the thalamic nuclei were made at .3 mm. intervals and the degeneration was analyzed by visual inspection and plotted. Criteria of degeneration in the thalamus were cell loss and glial reaction. No attempt was made to measure precisely the amount of cell loss.

RESULTS

The six brains can be conveniently divided into two groups: the first four ablations performed comprise the first group; the last two ablations, the second.

First Group

Cat ABLE: The lesion was limited to the left hemisphere and had as its bounds: laterally, the rhinal fissure; anteriorly, the posterior orbital gyrus proreus; posteriorly, the tip of the pyriform lobe; medially, the orbito-frontal and antero-limbic cortex. The ablation removed most of the

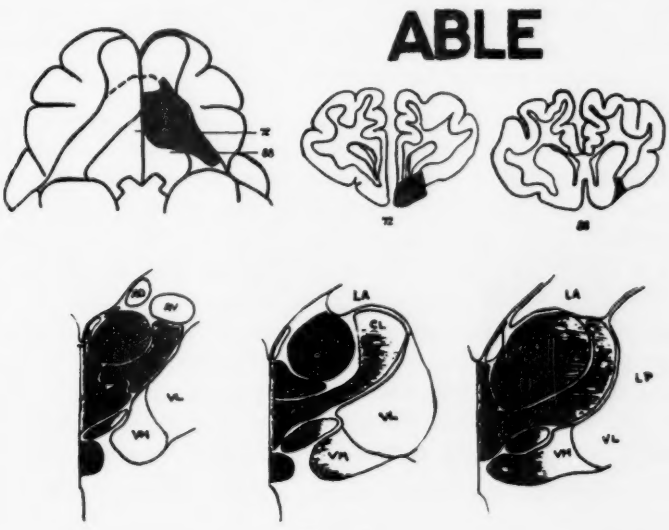

PLATE 1.

PLATES 1-4. Schematic drawings of the lesions and thalamic degeneration in all animals, including a composite drawing of the first group of animals (Plate 3). On Plate 3, the dotted area represents the cortical and subcortical structures involved by at least one lesion. The solid black areas show only the area common to all the lesions in the first group of animals and only the area of thalamic degeneration found in all animals of this group.

The thalamic nuclear delineation is based upon the atlas of V. Jimenez-Castellanos (*J. comp. Neurol.*, 1949, *91*, 307-330).

Abbreviations are as follows:

AD — n. antero dorsalis
AV — n. antero ventralis
CL — n. centralis lateralis
CM — n. centralis medialis
LA — n. lateralis anterior
LP — n. lateralis posterior
MD — n. medialis dorsalis
PC — n. paracentralis
RE — n. reuniens
RH — n. rhomboidialis
SM — n. submedius
VL — n. ventralis lateralis
VM — n. ventralis medialis

anterior olfactory tubercle, but none of the posterior tubercle and most of the prepyriform cortex. In depth, the most inferior tip of the head of the caudate nucleus and some of the most inferior fibers of the internal capsule were destroyed (Plate 1).

Thalamic degeneration was severe in n. centralis medialis, n. paracentralis, n. submedius, n. rhomboidialis, and the medial portions of n. medialis dorsalis; moderate in n. reuniens, n. centralis lateralis, the lateral portions of n. medialis dorsalis, and the medial portions of n. ventralis medialis; slight in the more anterior portions of n. ventralis medialis (Plate 1).

Cat CHARLIE: This unilateral lesion was deliberately more extensive than that in ABLE. It extended laterally beyond the anterior rhinal fissure and posteriorly into the pyriform lobe, involving the amygdala. It removed posterior olfactory tubercle and much prepyriform cortex, but did not touch orbito-medial cortex or the caudate nucleus. A few of the most inferior fibers of the internal capsule were interrupted, and a considerable portion of orbital gyrus proreus was ablated (Plate 2).

Severe thalamic degeneration was present in n. centralis medialis, n. paracentralis, n. submedius, n. rhomboidialis; moderate in most of n. ventralis medialis and in a few parts of n. medialis dorsalis; slight in n. centralis lateralis and in most of n. medialis dorsalis. No degeneration was present in n. reuniens and some parts of n. medialis dorsalis (Plate 2).

Cat EASY: This was another extensive lesion. It involved the orbital surface of gyrus proreus, anterior olfactory tubercle, prepyriform cortex, anterior portions of pyriform lobe, orbital gyrus, anterior ectosylvian gyrus, anterior sylvian gyrus, antero-limbic and infero-limbic cortex on the left. The lesion also extended across the midline and infringed slightly upon portions of the antero-limbic and infero-limbic cortex on the right. The head of the left caudate nucleus showed moderate atrophy, probably secondary to devascularization, while the inferior left internal capsule was partially ablated (Plate 2).

Thalamic degeneration was severe in n. reuniens, n. rhomboidialis, n. centralis medialis, n. paracentralis, n. submedius, n. ventralis medialis, and n. medialis dorsalis; moderate in n. centralis lateralis; slight in portions of n. centralis lateralis and of n. medialis dorsalis (Plate 2).

Cat FOX: The lesion in this animal was again restricted. It was unilateral, remaining entirely medial to the anterior rhinal fissure and involving anterior olfactory tubercle, prepyriform cortex, and very slight amounts of antero-limbic cortex. Portions of the head of the caudate and the inferior

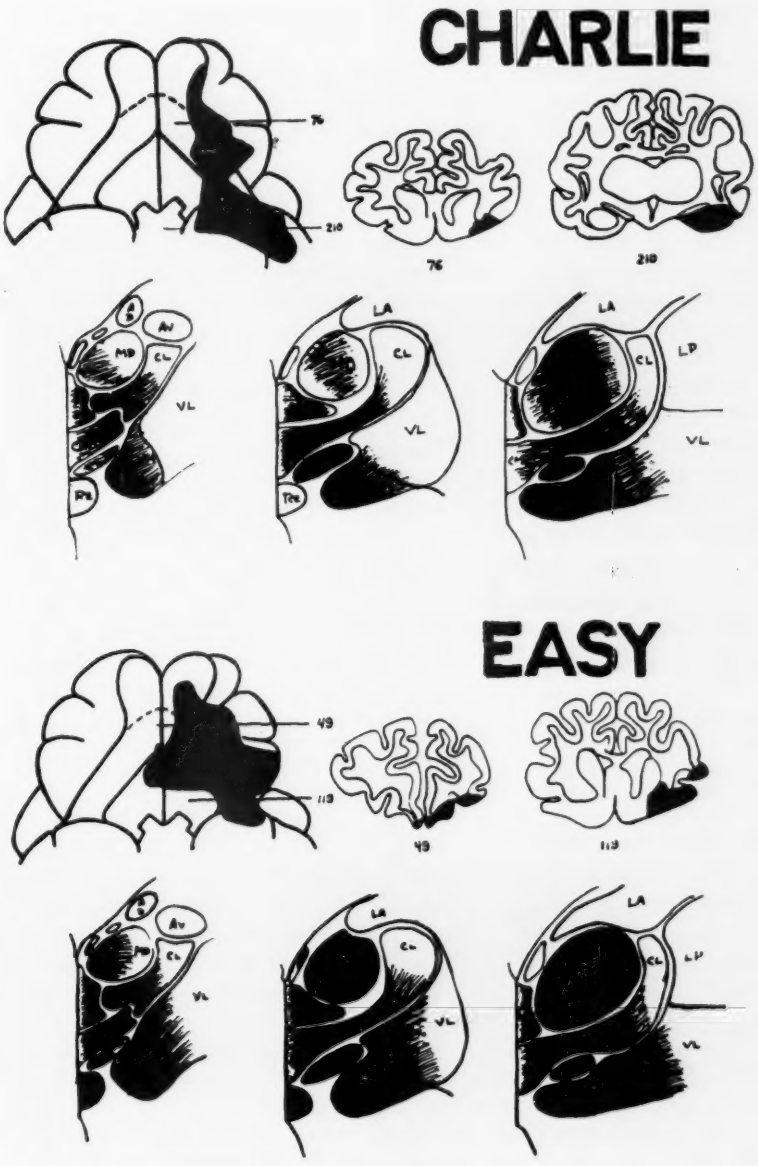
CHARLIE
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PLATE 2.

fibers of the internal capsule showed gliosis which was probably secondary to devascularization (Plate 3).

Thalamic degeneration was severe in n. reuniens, n. rhomboidialis, n. centralis medialis, n. paracentralis, n. submedius, and n. medialis dorsalis; moderate in some parts of n. ventralis medialis. No degeneration was present in n. centralis lateralis (Plate 3).

Second Group

Cat GEORGE: This entirely left-sided lesion was quite limited in extent, ablating orbital gyrus proreus, a small portion of the anterior olfactory tubercle and most of anterior prepyriform cortex. It did not extend lateral to the anterior rhinal fissure nor did it impinge upon medial surface cortex. A small amount of white matter subjacent to proreus was the only subcortical tissue involved by this lesion. A limited area in the region of the diagonal band appears to have been replaced by glial tissue, possibly secondary to devascularization (Plate 4).

The thalamic degeneration varied from moderate to slight in portions of n. centralis medialis, n. paracentralis, n. rhomboidialis, n. submedius, n. medialis dorsalis, and posterior portions of n. anterioris medialis (Plate 4).

Cat HARRY: In this, the most limited and superficial lesion of the series, only a very slight amount of posterior orbital gyrus proreus and a somewhat larger area of anterior prepyriform cortex on the left were removed. No other cortical or subcortical structures were involved (Plate 4).

There was minimal degeneration in anterior n. centralis medialis and n. paracentralis while none was present in any other thalamic nuclei, including n. medialis dorsalis (Plate 4).

DISCUSSION

Historical Background

Nissl,[21] and later Bard and Rioch[3] and Papez,[22] noted midline and intralaminar degeneration with large telencephalic and diencephalic lesions in the cat and dog. Waller[25] found n. centralis lateralis and n. reuniens cell loss in almost totally hemidecorticated cats. Although the caudate nucleus was damaged in all of Waller's material, no n. centralis medialis degeneration was found.

Using retrograde techniques, Rose and Woolsey[26] described midline and intralaminar degeneration following forebrain lesions which were more circumscribed than had been previously described. This fact, in view of the similarity between the phylogenetic development of the midline-intralaminar group and that of the allocortex, led them to postulate anterior

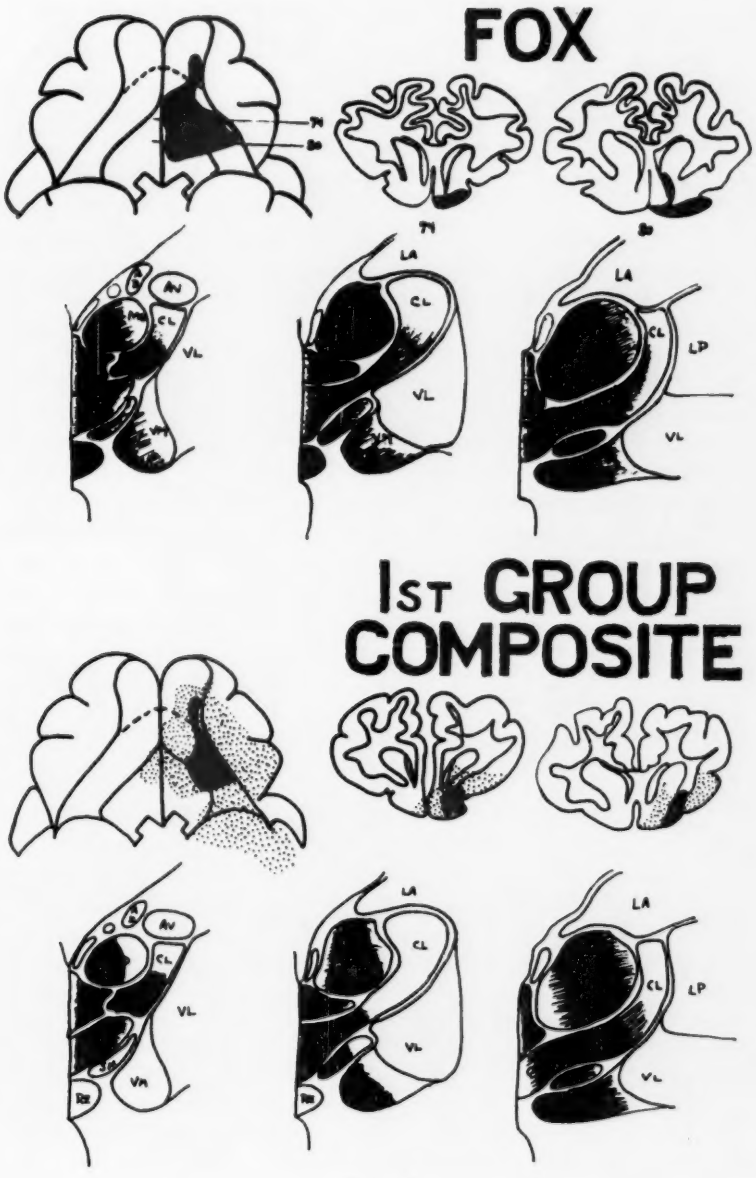

PLATE 3.

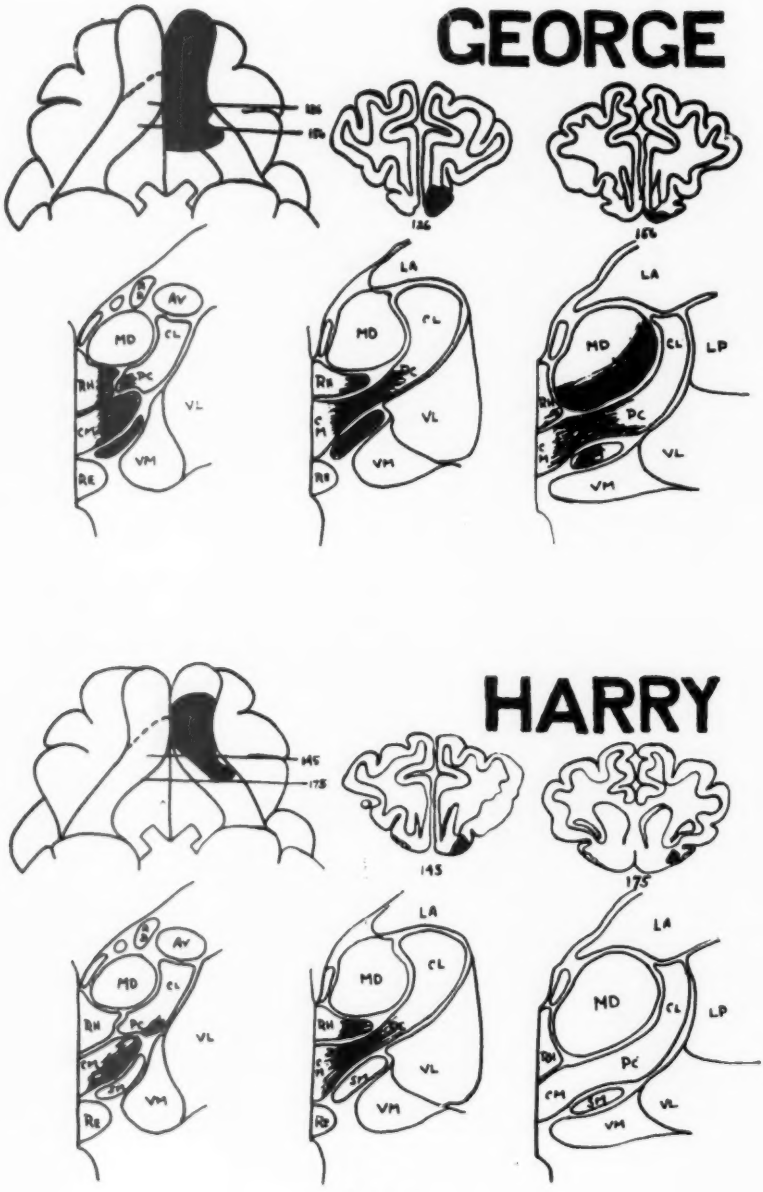

PLATE 4.

rhinencephalic projections from these nuclei. Anatomical analysis of bilateral fronto-temporal ablations in monkeys by Pribram and Bagshaw[26] and Bagshaw and Pribram[2] provided further suggestive evidence of such rhinencephalic projections.

Utilizing silver techniques and thalamic lesions, Nauta and Whitlock[20] found projections from rostral midline and intralaminar nuclei to "phylogenetically old cortex: prepyriform, infralimbic and limbic cortical area and to presubiculum and entorhinal areas."

Finally, Jasper[10] mentions that "there is some evidence that stimulation . . . of mesio-frontal and anterior limbic (cortical) areas may be able to . . . exert generalized effects upon other areas of cortex in both hemispheres."

On the other hand, Walker[34] found no evidence of midline and intralaminar degeneration in any of his cortical ablations, and Droogleever-Fortuyn[7] believes on the basis of his more restricted but still relatively extensive ablations that, with regard to the midline nuclei in the rabbit, "connections with the striate body seem more probable."

Mettler[14] described, on the basis of extensive neocortical ablations, "subcortical efferents" of unknown destination from these nuclei.

Powell and Cowan[24] state that the midline and intralaminar nuclear group of the rat have an independent projection to the cortex of the medial surface of the hemisphere and to the striatum. Later, Cowan and Powell[6] found that in a large series of rabbits n. centralis lateralis and the more medial intralaminar nuclei degenerated after lesions of the caudate nucleus and denied any cortical projection from these nuclei. A final study[25] of the thalamo-striate relationships in the monkey by these same authors seemed to confirm their findings in the rabbit.

Theoretical Formulations

Of the generally accepted thalamic components of the "central activating system," only two, the anterior and reticular nuclear groups, possess projections to neocortex, and of these two, only the reticular complex projects to a sufficiently large portion of the cortical mantle to be able to directly influence generalized cortical activity. However, as Rose[31] and Chow[5] have demonstrated, this projection is "distributed to the neocortex in a topographically specific manner" with the anterior reticular nucleus degenerating after only more medial cortical ablations. Therefore, since the great bulk of the activating system nuclei, i.e., the anterior and midline-intralaminar groups, do not possess isocortical efferents,[20, 30] they apparently produce the "recruiting response" by means of mechanisms other than a direct, one-neuron corticopetal pathway. On the basis of a somewhat sparse and

often contradictory neuro-anatomical literature concerning this region, there seem to be several alternative, though certainly not mutually exclusive, mechanisms possible.

The midline and intralaminar and anterior nuclei could modify cortical activity via the reticular complex of the thalamus, with neurons originating in the latter forming a sort of "final common path" for impulses from the more caudal portions of the "central activating system."[8, 21]

The midline-intralaminar and anterior nuclei might affect cortical activity on a subcortical level by means of intrathalamic interaction with the medial, ventral, lateral, and pulvinar groups.[28] It is also possible that the "central activating system" nuclei might act on the cortex by means of efferents synapsing in the medial, ventral, lateral, and pulvinar nuclei, with secondary neurons then passing to the specific projection area of each nucleus.

The basal ganglia might similarly function as mediators of impulses from the midline and intralaminar nuclei, although most anatomical studies have thus far failed to reveal significant numbers of striatocortical fibers.[4] Mettler *et al.* have, however, produced short-latency changes in neocortical electrical activity after caudate stimulation. These authors also describe a "decrease in size" of the caudate and a "loss of cells" in the caudate after cortical ablations.[15]

Finally, the midline and intralaminar nuclei may project to the orbito-frontal allo- and juxtallocortex of the first and second rhinencephalic systems,* paralleling the projection of the anterior nucleus to the cingulate juxtallocortex of the third rhinencephalic system.*[27] This orbito-frontal cortex would then function as a part of the "central activating system" by elaborating and transmitting midline-intralaminar activity to neocortex via intracortical and cortico-cortical tracts.[20]

Of these possible mechanisms, the latter two have seemed most reasonable, in view of the long latency of the recruiting response, 20-35 msec.,[19] and the apparent independence of the response from neocorticothalamic

* The "rhinencephalon" has been defined by Pribram and Kruger as "including all cortical formations not typically isocortical, i.e., those which do not definitely pass through a six layer stage in ontogeny," thus including both allocortical and juxtallocortical formations. Further, on ontogenetic and experimental anatomical grounds, they have grouped these formations into three systems: the first system is semicortical (M. Rose), has direct connections with the olfactory bulb and includes the olfactory tubercle, prepyriform cortex, area of the diagonal band, and the corticomedial nuclei of the amygdala; the second system is perisemicortical and subcortical, has direct connections with the first system, and includes the subcallosal and fronto-temporal juxtallocortex, the septal nuclei and the basolateral nuclei of the amygdala; the third system is archi- and periarchicortical, has direct connections with the second system but none with the first system or bulb, and includes Ammon's formation, the entorhinal, retrosplenial, and cingulate juxtallocortex.[27]

reverberating circuits.[16] However, opinion has been sharply divided as to whether the basal ganglia or the anterior rhinencephalic cortex receives the principal projection of the midline and intralaminar nuclei.

SUMMARY AND CONCLUSIONS

The lesion common to all animals of the first group, ABLE, CHARLIE, EASY, and FOX, included prepyriform cortex and the orbital surface of gyrus proreus. The only nuclei which degenerated in all four of these brains were: nuclei centralis medialis (CM), rhomboidialis (Rhomb), paracentralis (PC), ventralis medialis (VM), and medialis dorsalis (MD). As reported earlier,[18] on this basis it was felt that the evidence was indicative, though not conclusive, that the midline and medial intralaminar nuclei project to anterior rhinencephalic cortex, and that the more lateral intralaminar nuclei project elsewhere.

However, because of the slight subcortical injuries found in all of the first section of animals (Plate 3), additional, more circumscribed, ablations were performed in an attempt to provide material less subject to controversial interpretation. The second group, which comprises cats GEORGE and HARRY, represents this attempt.

These latter two lesions differ only as to the total amount of cortex removed and are similar in that no appreciable subcortical damage is present in either.

In GEORGE, in which a relatively large amount of gyrus proreus and prepyriform cortex is removed, the midline and paracentral degeneration is moderate in degree and quite well-defined in extent. The diffuse pattern of the moderate degree of n. medialis dorsalis cell loss is compatible with the slight interruption of the subcortical fibers to the gyrus proreus. There is no possibility that the internal capsule or the basal ganglia are involved by the lesion (Plates 4, 7).

HARRY presents minimal prepyriform destruction and, quite understandably, the thalamic degeneration is correspondingly minimal. There is definite cell loss in the more anterior portions of the midline and paracentral nuclei, while slight changes are to be noted in n. medialis dorsalis and none in any other thalamic nuclei (Plates 4, 5, 6).

The almost constant severe degeneration in the puzzling n. submedius still remains unexplained, but probably indicates a projection to the cortex within the rhinal fissure.

Therefore, within the limits of the retrograde degeneration technique, the second group of preparations demonstrates, in a more refined and pre-

cise fashion than that of the first, the existence of efferents from the midline and medial intralaminar nuclei to prepyriform cortex.

Needless to say, these findings do not exclude the possibility of other efferents from these nuclei, nor do they indicate possible connections of the more lateral and posterior portions of the intralaminar group of nuclei. Indeed, the more extensive lesions of the first section of brains seem to

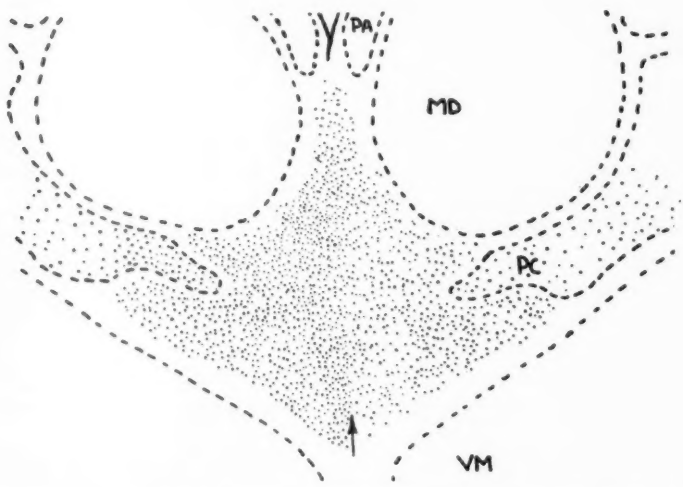

PLATE 5. A drawing of a low-power photomicrograph of Harry (Section #320) x45. Arrows delineate the midline. Retrograde cell loss on right side of drawing involving CM and medial PC.

indicate that the major projection areas of n. centralis lateralis, lateral n. paracentralis, and n. centrum medianum do not include the prepyriform cortex. This would be quite compatible with the work of Powell and Cowan[9, 34, 35] in which, after striatum lesions, the more severe degeneration seemed to be more laterally situated than in our material. Further, in our most extensive ablations, there was never degeneration of more than half of the cells in even the more medial nuclei of the midline and intralaminar group, suggesting the possibility of an additional projection elsewhere of the entire nuclear group.

The results of this study suggest, therefore, that all of the thalamic nuclei from which the recruiting response can be elicited project to the rhinencephalon and very closely related striatal structures. The superior midline and medial intralaminar nuclei, n. rhomboidialis, n. centralis medialis, and

n. paracentralis, project to the prepyriform allocortex of the first rhinencephalic system. There is considerable evidence that the other major midline nucleus, n. reuniens, projects to the infralimbic cortex, i.e., the subcallosal juxtallocortex of the second rhinencephalic system.[6, 7, 11, 25, 29] Finally, the anterior nucleus projects to the cingulate juxtallocortex of the third rhinencephalic system. Thus, a close relationship is established between these centrally located thalamic nuclei.

ACKNOWLEDGMENT

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THE EPIDEMIOLOGY OF ASEPTIC MENINGITIS AND RELATED NONSPECIFIC DISEASE IN CONNECTICUT, 1957: VIROLOGICAL AND CLINICAL STUDIES†

INTRODUCTION

The advent of modern tissue culture techniques reported in 1949 by Enders, Weller, and Robbins[7] initiated a new era in the study of virus infections. As a result, it has been possible to carry out systematic studies of the etiology of disease syndromes, and, to date, some seventy new agents have been isolated. It is now possible for virus isolation to contribute to the establishment of an etiological diagnosis in cases of poliomyelitis and aseptic meningitis for which previously there had been no practicable laboratory evidence of disease.[1, 8]

The accidental discovery in tissue culture of the Echo group of viruses was followed by the demonstration that these agents are widely distributed in normal populations.[9, 10, 21] It has now become apparent that in addition to sharing epidemiological similarities with Coxsackie and polioviruses, members of the Echo group are also capable of producing characteristic disease patterns.[4]

Not until 1954 was the association of Echo viruses with aseptic meningitis firmly established, when outbreaks associated with type 6 were described.[5, 14] Subsequently type 4[3, 13] has also been reported as cause of extensive outbreaks of aseptic meningitis. During 1956, for the first time, numerous epidemics associated with rash were reported, the etiologic agent being Echo Virus Type 9.[19, 28] In these outbreaks many associated cases of nonspecific febrile illness with rash also were reported.

In Connecticut, Davis and Melnick[3] carried out studies covering 1955 and 1956 to determine the etiology of reported cases of aseptic meningitis

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and paralytic poliomyelitis. The purpose of the present study was to investigate the activity of the Enteroviruses—Echo, Coxsackie, and polioviruses—as causative agents of aseptic meningitis and associated nonspecific illnesses in Connecticut in 1957 and to compare the results with those of the previous two years.

MATERIALS AND METHODS

Source of Materials

Fifty-seven cases of suspected poliomyelitis, aseptic meningitis, or related nonspecific febrile illness, occurring in Connecticut from June through September, 1957, were studied. Specimens collected from these patients were sent to the laboratory by physicians throughout the state.* An attempt was made to obtain stool, throat swabs, cerebrospinal fluid, and blood specimens in the acute phase of disease from as many cases as possible. Convalescent blood specimens were collected approximately three weeks after the onset of illness.

Virus Isolation Techniques

Rhesus monkey kidney (MK) tissue culture monolayers were used routinely for viral isolation. These were prepared in the standard manner for the laboratory, i.e., primary trypsinized MK cells grown in stationary tubes in a medium consisting of Hanks balanced salt solution with 2 per cent calf serum and 0.5 per cent lactalbumin hydrolysate.[18]

Stool specimens were made into 20 per cent suspensions with distilled water spun for one hour at 3,000 RPM. The supernatant fluid, after treatment with antibiotics (500 units penicillin, 500 micrograms streptomycin, and 25 micrograms of mycostatin per ml.), served as inocula: each of five monkey kidney tubes drained of media was inoculated with one ml. of stool suspension and incubated at 37° C. for one hour. The inocula were then discarded and the fluid nutrient medium replaced.

Throat swabs were extracted with 2 ml. of Hanks balanced salt solution containing antibiotics, centrifuged at 3,000 RPM for 30 minutes and inoculated in 0.2 ml. amounts into each of four tubes.

Cerebrospinal fluid was inoculated directly in 0.2 ml. amounts into each of three tubes.

All cultures were read daily or at least at two-day intervals. Those showing cytopathic effect (CPE) were harvested and passed. Negative cultures were discarded after 14 days.

Virus Identification

The use of *Erythrocebus patas* monkey kidney cells as a screening procedure for grouping Enteroviruses has been described by Hsiung and Melnick.[11] Through the courtesy of Dr. Hsiung, it was possible to use patas cultures to facilitate identification. Strains which produced CPE in patas cells and those which failed to do so were set up in neutralization tests against the appropriate pools of Coxsackie, Echo, and poliovirus hyperimmune monkey antisera. As an additional aid to identification, and to rule out the

* We are grateful to all the co-operating physicians and particularly to Dr. Joyce V. Deutsch, Bristol, Connecticut, for her generous assistance.

possibility of virus mixtures in dealing with untypable agents, plaque morphology of certain virus strains was studied in rhesus MK monolayers under agar.[13]

Serological Tests

Typing of virus strains was carried out using 100 TCD_{50} of virus in 0.1 ml. volume against the appropriate serum pool. In each test the sera were used in dilutions such that approximately 20 units of antibody were present per 0.1 ml. The tests were incubated for one hour at room temperature and 0.2 ml. of the resultant virus-antisera mixture was inoculated into each of four rhesus MK tubes.

If neutralization occurred with one of the test pools as noted by inhibition of cytopathic changes, a similar neutralization test was set up with the individual types of specific antisera present within the pool.

Neutralizing antibody tests against Echo and Coxsackie viruses. The acute and convalescent sera (when available) obtained from virus positive cases and contacts were set up in the same neutralization test. Dilutions of 1:10, 1:50, and 1:250 of sera were used against 100 TCD_{50} of a specific prototype virus. Certain paired sera in similar dilutions were challenged with virus isolated from cases from which the sera were obtained.

Poliomyelitis antibodies. Poliomyelitis complement-fixing (CF) antibody determinations were carried out in plastic plates according to the method of Black and Melnick.[3] Both unheated policmyelitis type I antigen and heated pooled poliomyelitis types I, II, and III antigens were used. Poliomyelitis neutralizing antibody studies were performed in disposable plastic panels according to the method of Melnick and Opton[17] with poliovirus types I, II, and III.

Mumps complement-fixing antibody. In selected cases of aseptic meningitis where there was evidence of possible exposure to mumps virus, the acute and convalescent sera were submitted to the Department of Laboratories of the State of Connecticut for mumps complement fixation tests.

Animal Tests

All strains of Echo Virus Type 9 isolated and all strains of virus which were not neutralized by any of the antisera were tested for pathogenicity in suckling mice. Animals between one and three days of age were inoculated with 0.02 ml. subcutaneously and 0.01 ml. intracerebrally using high titered (at least 10^6 TCD_{50}) first or second tissue culture passages. The mice were observed daily; any showing signs of paralysis were harvested, and in some instances histological sections were made. In the latter event, the involved limb was fixed in 95 per cent Zenker's fluid plus 5 per cent acetic acid; sections were stained with hemotoxylin and eosin.

RESULTS

Virus isolations. Of the 57 cases of suspected poliomyelitis, aseptic meningitis, or related nonspecific febrile illness studied, some virus was isolated from 33, or 58 per cent. The specific agents are shown in Table 1.

A striking feature of the results was the paucity of isolations of poliovirus: only 2 strains, one type I from a five-year-old nonparalytic case from

Putnam, and another type I from a paralytic case, aged 18 months, from Derby. Both children had received Salk vaccine, three and two doses respectively. The last dose had been given seven months prior to the onset of illness in the nonparalytic case and four months prior to onset in the paralytic case.

TABLE 1. VIRAL ISOLATIONS AND TYPE OF ILLNESS

Virus	*Aseptic meningitis*	*Febrile illness*	*Paralytic poliomyelitis*	*% of total virus isolations*
Polio I	1	0	1	6%
Echo 9	8	5	0	40%
Echo 14	1	0	0	3%
Coxsackie A-9	0	3	0	9%
Coxsackie B-3	1	0	0	3%
Coxsackie B-4	2	0	0	6%
Coxsackie B-5	1	1	0	6%
Unidentified†	4	5	0	27%
Mumps*	5	0	0	
None	15	4	0	
Total	38	18	1	
% Diagnosed Etiologically	61	78		

* No virus isolated but rise from 0-64 in mumps CF test using acute and convalescent sera.

† Unidentified viruses were not typable with antisera against the known types of Echo, Coxsackie, and polioviruses.

Sources of virus. Stools proved to be the best source of viral isolation, though cerebrospinal fluid yielded a significant number of positives for Echo Virus Type 9. Table 2 depicts sites of virus isolation in this study.

Viral properties. The results with patas cells and plaque morphology of the freshly isolated strains of the recognized viruses corresponded well with the results with prototype strains reported by Hsiung and Melnick.[11, 13] Unlike the prototype Hill and Quigley strains of Echo 9 virus, the strains isolated in Connecticut in 1957 caused paralysis in suckling mice. The histological lesion was a myositis indistinguishable from that characteristic of Coxsackie A virus infections. This is similar to the experience with European strains of Echo 9 in 1956.[15] None of the untypable agents produced disease in suckling mice.

Serological results. The results of neutralizing antibody studies on available sera (Table 3) indicate that the cases of infection with Echo Virus Types 9 and 14, and Coxsackie B, showed at least a fivefold increase in neutralizing antibody from the acute to the convalescent serum specimens. In the two instances in which antisera from the patient were set up against

TABLE 2. SOURCES OF VIRAL ISOLATION

	Echo 9	*Echo 14*	*Coxsackie*	*Poliovirus*
Stool	11/13*	1/1	8/8	2/2
Throat	3/5	0/0	2/3	1/2
CSF	4/7	0/0	0/1	0/0

* Denominator indicates the number tested; numerator indicates the number positive.

TABLE 3. SEROLOGICAL RESULTS

		Neutralizing antibodies†	
Case	*Virus*	*Acute*	*Convalescent*
7-63	Echo 9	0	10
7-70*	Echo 9	50	250
7-91	Echo 9	0	250
7-101*	Echo 9	10	50
7-103	Echo 9	0	50
7-105	Echo 9	0	250
875	Echo 9	0	250
7-54	Echo 14	0	10
7-38	Coxsackie B-3	10	50
7-55	Coxsackie B-4	0	10
7-61	Coxsackie B-4	10	50
7-93	Coxsackie B-5	0	50

* In these cases sera was also tested with the virus isolated from the sick individual and proved to have the same level of antibodies as with the prototype virus.

† Numbers represent reciprocals of serum dilutions which neutralized 100 TCD_{50} of virus.

his own virus, the degree of antibody rise was similar to that found when the prototype strain was used.

All available sera, from both virus positive and virus negative cases, were tested for poliomyelitis CF and neutralizing antibodies. None of the virus negative cases showed evidence of recent poliovirus infection. Except for the patients with poliomyelitis, in none of the virus positive cases was there any indication of concurrent infection with poliovirus. Serological

studies of the paralytic case due to poliovirus type I showed high type I CF levels (>32) in both acute and convalescent specimens, and a rise in type I neutralizing antibody between acute and convalescent specimens.

Five cases which failed to yield a virus showed a rise from O in acute to 64 in the convalescent serum specimens in the mumps CF test.

TABLE 4. DISTRIBUTION OF VIRUS POSITIVE CASES IN CONNECTICUT

Virus isolation	*Location*	*No. of cases*
Poliovirus I	Derby	1
	Putnam	1
Coxsackie A-9	Bristol	3
Coxsackie B-3	Greenwich	1
Coxsackie B-4	Greenwich	1
	Berlin	1
Coxsackie B-5	Essex	2
Echo 9	Bristol	9
	Berlin	1
	Danielson	1
	Hartford	1
	Willimantic	1
Echo 14	Newington	1
Unidentified	Bristol	7
	Norwalk	1
	Stamford	1

Geographical Location of Cases

Table 4 indicates the distribution of virus positive cases in Connecticut. Since the specimens were submitted for analysis by physicians on a voluntary basis, it is not possible to draw conclusions about the actual prevalence of the agents isolated. However, it seems clear that in the town of Bristol, Echo 9 and several other viruses were active simultaneously, for 19 of the 57 virus positive cases resided there; 9 of the infections were due to Echo 9, 3 to Coxsackie A-9, and 7 belonged to the group of untypable agents.

In the rest of the state, both virus positive and virus negative cases were widely distributed. The two poliovirus isolations were from cases from Derby and Putnam. Cases of aseptic meningitis due to mumps occurred in Bristol, Bridgeport, Waterbury, and Meriden. None of the virus positive cases lived in the New Haven area.

Clinical Characteristics

Infections with Echo Virus Type 9. Eight of the 13 patients infected with this virus presented the picture of aseptic meningitis, and five had nonspecific febrile disease (Table 1). Aside from the presence of meningeal signs in the former, there were no significant differences in the two

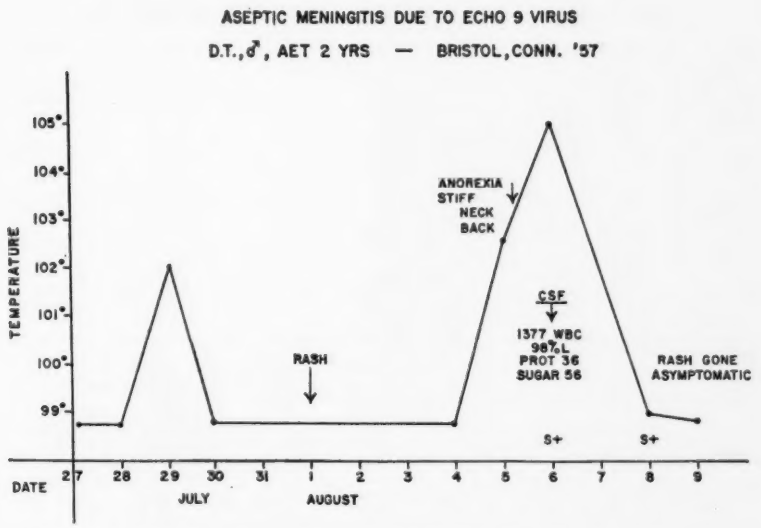

FIG. 1. S+ indicates stool positive for Echo 9 virus. *N.B.* The CSF was positive for Echo 9 virus in this case.

groups. The most common symptoms were fever, headache, nausea, vomiting, and, particularly in young children, rash. Of the eight with aseptic meningitis, only three had Kernig and Brudzinski reflexes and one had transient leg weakness. The duration of illness in those without meningeal signs averaged 2-4 days while in the aseptic meningitis cases it was 4-6 days.

The most unusual feature of the clinical picture was the presence of an exanthem in five of the 13 individuals. No exanthem was noted in any of these. There was an association between age and the presence of rash: all of the virus positive children under four years of age (five in number) had a rash while in none of the older children (six in number) and neither of the two adults who were infected was rash detected. In addition, three other children under four years of age, who were members of families with

virus positive members but from whom no specimens had been obtained, exhibited rash. The rash was similar in character and distribution to that of rubella and was in fact often mistaken for rubella. The lesions were maculopapular and erythematous; in some children they extended over the face, trunk, and extremities, while in others they were limited to the face or

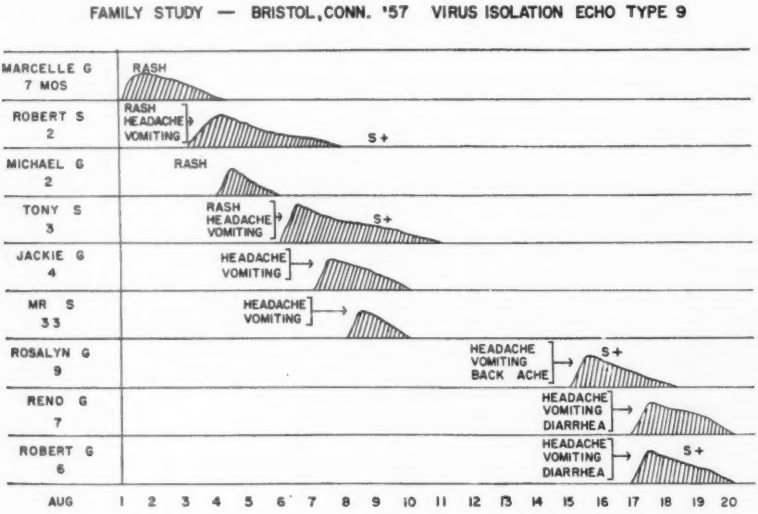

FIG. 2. Crosshatched areas indicate duration of fever. S+ indicates stool positive for Echo 9 virus.

trunk. The rash lasted approximately three to five days but on occasion as long as eight days. It appeared both before and after the onset of fever.

Figure 1 shows diagrammatically the course of illness in a two-year-old boy. This child had one day of fever followed by five days of well-being, after which the fever returned and was accompanied by meningeal signs. The characteristic rash made its appearance three days before the onset of the second bout of fever and persisted for eight days. The biphasic course in this case was unusual for this series, but it has been reported in up to 40 per cent of cases in an outbreak of Echo 9 infection in England;[24] a third phase has also been noted in a small per cent of children but was not seen in any of the Connecticut cases studied in 1957.

The family epidemiology of Echo 9 infections is illustrated in Figure 2. This family consisted of two—the G and the S families—who lived together

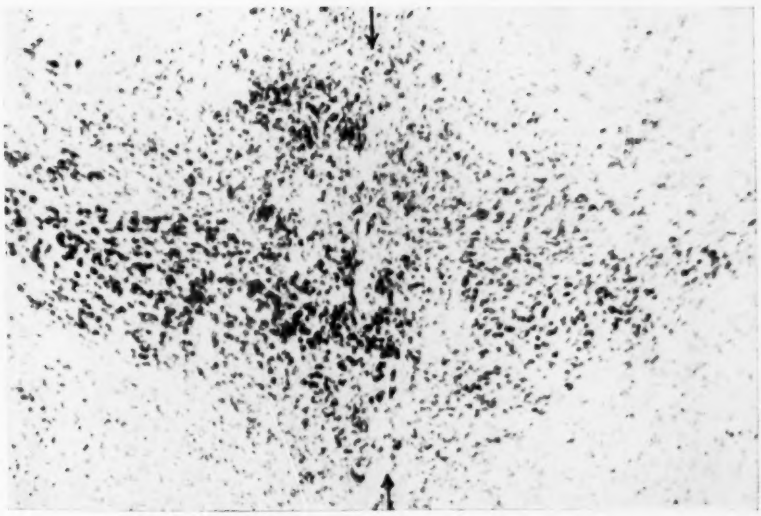

PLATE 6. A photomicrograph of Harry (Section #320) x70. Arrows delineate the midline. Retrograde cell loss and glial proliferation on the right side of the picture, involving CM and medial PC. Thionine blue stain.

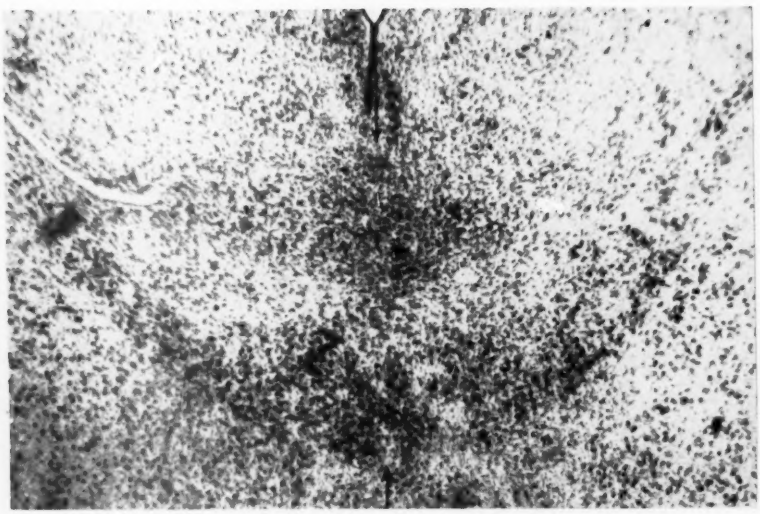

PLATE 7. Photomicrograph of George (Section #336) x45. Arrows delineate the midline. Moderate retrograde cell loss and glial proliferation on the right side of the picture, involving CM, medial PC, submedius and slight portions of Rhomb. Thionine blue stain.

in one home. All of the eight children were ill, and one of the four adults had a febrile illness. The young children were the first to be affected, and four of the five children four years and younger developed the characteristic rash; the older children and the one parent involved had similar symptoms but no rash. There were no frank cases of aseptic meningitis in any mem-

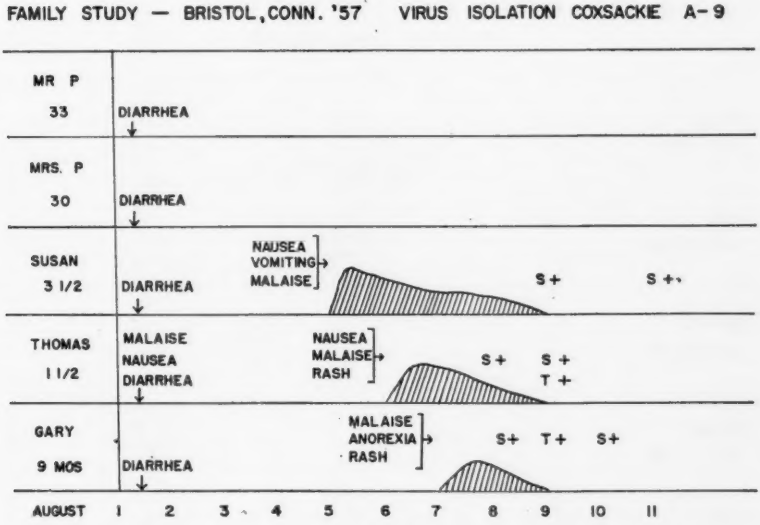

FIG. 3. Crosshatched areas indicate duration of fever. S+ indicates stool positive for Coxsackie A-9 virus. T+ indicates throat swab positive for Coxsackie A-9 virus.

bers of this household, but since no spinal punctures were done, it is not possible to rule out meningeal involvement.

Cerebrospinal fluids were obtained from eight cases with Echo 9 infection. All of these showed a pleocytosis ranging from 10 to 1377 WBC/mm^3 with an average of 429 and a median of 311. Two cases had more than 50 per cent polymorphonuclear leucocytes, while all others showed a great lymphocytic preponderance. Spinal fluid protein ranged from 26 to 62 mg % with an average of 42.5 and a median of 39.5; and only three were elevated at levels greater than 45 mg %.

Infections with Coxsackie A-9. Three children under 3½ years of age presented with nonspecific febrile disease, and from them Coxsackie A-9 virus was isolated. The clinical picture was similar to that of the Echo 9 cases and was characterized by fever, nausea, vomiting, and rash. The

rash was seen in two of these cases and as with the Echo 9 cases was a spotty erythematous papular eruption on the face, trunk, and extremities. The rash appeared one day prior to the fever and persisted approximately four days as depicted in Figure 3. The presence of diarrhea in all members

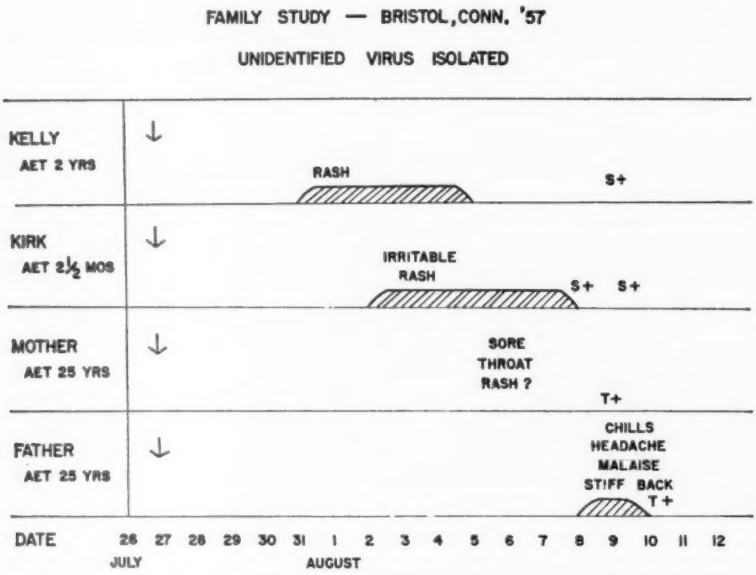

FIG. 4. Crosshatched areas indicate duration of fever. S+ indicates stool positive for unidentified virus. T+ indicates throat swab positive for unidentified virus.

of this family five days before the onset may or may not have been associated with Coxsackie A-9 infection.

Infections with unidentified viruses in Bristol. Clinically, these seven cases all had pyrexia and in general a milder disease process than that seen in the Echo 9 and Coxsackie A-9 cases in Bristol. Six of the cases, all in children under the age of seven years, had a rash which did not differ in appearance or distribution from the rash seen in the Echo 9 and the Coxsackie A-9 cases. There was a tendency for it to become more confluent after approximately two days and it gradually faded in approximately one week. In addition to fever and rash, two of the children had headache, stiff neck, and malaise; a spinal fluid was obtained from one of these cases

and showed a pleocytosis of 110 cells, all lymphocytes, with a protein value of 24 mg %. The others merely had fever and rash.

The one adult case was a 27-year-old father who had no rash but did develop chills, fever, retro-orbital pain, sore throat, and lethargy of two days' duration. Virus was isolated from his throat on the third day of illness.

Figure 4 is a representative family study of disease caused by an unidentified Bristol virus.

DISCUSSION

The etiology of the disease syndromes under study were considerably different in 1957 from the two previous years. Whereas Davis and Melnick[9] had found that polioviruses accounted for 35 per cent of the 98 virus positive cases of aseptic meningitis in 1955 and 1956, in only one instance was a poliovirus (type I) isolated from a similar case in 1957. The types of Echo virus involved were also different, Echo 6 being the predominant one in the earlier two years with no isolations of Echo 9, while in 1957, Echo 9 was the most commonly isolated agent, and Echo 6 was not encountered. This shift from year to year has been a common feature in the short history of Echo virus infections.

There were also far fewer cases of aseptic meningitis due to any cause in Connecticut in 1957 compared to the preceding few years. However, a number of viral agents were active and probably were associated with a considerable amount of minor symptomatology. Since no illness surveys were conducted, it is not possible to say how much disease was produced by these various agents in the areas studied, but reports from physicians in the town of Bristol, for instance, suggest that a great deal of minor illness existed, particularly among the childhood population during the period in which the cases reported were studied. It seems likely that much of this illness was due to Echo Virus Type 9, as well as Coxsackie A-9, and other agents which could not be classified.

Clinically, the cases of aseptic meningitis of proven viral etiology showed a certain amount of minor variability but fulfilled the basic criteria established for the disease syndrome by Wallgren in 1925.[25] The outstanding unusual clinical finding, however, was the presence of rash in certain cases.

Rash had been reported associated with aseptic meningitis as early as 1946 by Gabinus[13] in Sweden. Parry and Good[20] reported similar findings in England in 1948. However, no etiologic agent was established for the disease process in either outbreak. Recent epidemics of aseptic meningitis associated with rash occurred in Europe in 1956, and Echo 9 virus was shown to be the commonest etiologic agent.[19, 23] In a Swedish epidemic[13] in

the same year, Echo 4 virus was associated with a similar picture, but in the large Echo 4 outbreak in Marshalltown, Iowa, in 1955, rash was not observed in any of the cases.[8]

In the outbreaks of aseptic meningitis associated with rash, many associated cases of nonspecific febrile illness with rash also have been reported. There also have been outbreaks of so-called "Boston Exanthem" disease reported by Neva,[18] in which febrile illness with rash was the main clinical form and aseptic meningitis cases did not occur. The Boston Exanthem has recently been shown to be due to Echo Virus Type 16.[4] None of the Connecticut viruses was neutralized by Echo Virus Type 16 antisera.

The association of rash with nonspecific febrile disease due to Coxsackie Virus Type A-9 seems to be an unusual experience, although there was an unreported case of aseptic meningitis associated with a mild rash due to Coxsackie Virus Type A-9 in Grace-New Haven Community Hospital in 1956,[28] and Sabin observed similar cases in 1957.[25]

SUMMARY

During the summer of 1957, virological and serological investigations were carried out on specimens from 56 patients in Connecticut diagnosed nonparalytic poliomyelitis, aseptic meningitis, or nonspecific febrile illness, and from one with paralytic poliomyelitis. It was possible to make a specific etiologic diagnosis on 33 or 58 per cent of the patients.

The absence of polioviruses as the cause of significant illness in Connecticut in 1957 was striking. Only two isolations of type I virus were made. This experience is in line with what occurred in the rest of the United States and is markedly different from the experience of the preceding years.

Echo and Coxsackie viruses played a definite role as the etiologic agents of aseptic meningitis and associated nonspecific febrile diseases in various parts of Connecticut during the summer of 1957. In addition, agents not belonging to any of the known Enterovirus types were also active in the same area at the same time and were associated with clinical syndromes similar to those produced by Echo and Coxsackie viruses. Echo Virus Type 9 was the most commonly isolated Enterovirus, a finding which differs markedly from the Connecticut experience of the previous two years.

An unusual clinical feature of the cases studied was the presence of a rubella-like rash, associated not only with Echo 9 virus infection, but with Coxsackie A-9 and with unidentified agents as well. The rash was similar in character and distribution in the three etiologic groups of cases.

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PLASMA LEVELS OF PROGESTIN DURING ABSORPTION OF PROGESTERONE PELLETS IN MICE†

Prolonged, continuous absorption of a steroid hormone can be readily accomplished by implantation of the hormone in the form of a compressed pellet or tablet. This study was undertaken in order to determine what plasma levels of progestin result when progesterone is absorbed continuously over long periods.

PROCEDURE

Pure progesterone, without excipient, was compressed into cylindrical pellets according to a standard procedure whereby each pellet has the same diameter and each is subjected to the same amount of compression.[3] The pellets were roughly 1 mm. in diameter, 6 mm. long, and 10 mgm. in weight. Some pellets were divided by cutting across the long axis. Prior to implantation each pellet was cleaned with a fine brush to remove any loose particles and weighed. The pellet was then placed in the lumen of a #13 hypodermic needle, the needle was pushed at least 1 cm. under the dorsal skin of the recipient, and the pellet was discharged with a stylet. The recipients, lightly anesthetized with ether at the time of implantation, were 90 mice of the inbred CHI, BC, A, and CBA strains. The mice had been ovariectomized 16 days before implantation of the pellets and were about 8 weeks old at the time of ovariectomy. Nine of the 90 mice died between implantation and autopsy or for other reasons could not be used.

One pellet, ranging in weight from 9.5 to 10.4 mgm., was implanted in each of 37 mice (Group 1).

One-half pellet (4.8-5.4 mgm.) was implanted in each of 30 mice (Group 2).

Two pellets (19.7-20.0 mgm.) were implanted in each of 14 mice (Group 3).

After various time intervals the mice were exsanguinated by dividing the carotid sheath under ether anesthesia. Mice in Groups 1 and 2 were bled at 2- to 5-day intervals; in Group 3, at 7-day intervals. Blood (0.6-1.2 ml.) from each mouse was received into a centrifuge tube containing 0.05 ml. of 20 per cent sodium citrate solution as an anti-coagulant and was centrifuged. The plasma was bio-assayed for "free" and "bound" progestin according to a standard procedure.[5,6] Since this assay does not differentiate between progesterone and its biologically active metabolites, Δ^4-3-ketopregnene-20α-ol and Δ^4-3-ketopregnene-20β-ol,[8,9] the material assayed is referred to collectively as "progestin." Activity is expressed in Progesterone Equivalents (P.E.);

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one P.E. is defined as equal to the *activity* of 1 μg of progesterone when the latter is assayed by the procedure of Hooker and Forbes in the absence of estrogen and other interfering factors.[8]

The pellet or pellets were recovered, cleaned of adherent material, dried in a desiccator, and re-weighed so that the per cent loss of weight could be determined. Connective tissue capsules always formed within a few days around each pellet. In three mice, the capsule at autopsy was filled with pus; in all other cases there was no macroscopic evidence of infection. One pellet by mistake was implanted intraperitoneally, but showed no difference in absorption rate.

RESULTS

When the per cent loss of weight of the pellets was plotted against time, the values up to the 90 per cent level fell close to a straight line, indicating a steady rate of pellet absorption. The 90 per cent was reached in approximately 55 days; subsequent values were ignored, as the residual pellets ultimately fragmented, and the rate of pellet absorption thereafter was no longer steady.

Plasma levels of "free" progestin in all three groups of animals rose to between 3 and 4 P.E./ml. plasma within a few days, then declined and fluctuated irregularly and without apparent pattern. The range for the 5 mgm. group was 0.4-3.6 P.E./ml. plasma; for the 10 mgm. group, 0.3-3.9 P.E./ml.; for the 20 mgm. group, 0.2-3.6 P.E./ml. In all three groups, "bound" progestin values also fluctuated irregularly, ranging as high as 1.8 P.E./ml. Mean values were determined, with the following results:

	"Free" Progestin			*"Bound" Progestin*		
Dosage group	*No. of mice*	*Mean*	*Standard error of mean*	*No. of mice*	*Mean*	*Standard error of mean*
5 mgm.	22	1.28	0.17	17	0.58	0.09
10 mgm.	20	2.04	0.23	15	0.64	0.12
20 mgm.	12	2.16	0.30	11	0.44	0.07

For the "free" fraction, the differences between the means for the 5 and the 10 mgm. groups and also for the 5 and the 20 mgm. groups have P values of less than 0.05 and are judged to be significant. The difference between the 10 and 20 mgm. groups is not statistically significant. Mean differences for "bound" progestin values when compared as groups also are not significant.

DISCUSSION

Deanesley and Parkes[1] pointed out long ago that the surface area is a major factor in the rate of absorption of a tablet of sex hormone. Forbes[8]

and others have confirmed this. When cylindrical pellets of progesterone were implanted subcutaneously in rats, then recovered and weighed, and when the per cent losses in weight were plotted against time, the values fell along a slightly bowed line.[2] Values for the three groups of mice fall along a line of the same shape. In both experiments the pellets were made and implanted in exactly the same way, employing the same apparatus, except that the pellets were placed in a ventral site in the rats and a dorsal site in the mice; 90 per cent absorption in the rats required 88 days, but in the mice about 55 days.

In view of a fairly steady absorption rate up to the 90 per cent level, it is curious that both "free" and "bound" plasma progestin levels fluctuate rather widely. This fluctuation perhaps is due to variation among mice of the different strains used or to variation in the rate at which the hormone is transported and disposed of in any mouse.

The highest levels of endogenous "free" progestin thus far detected in mice were 8-9 P.E./ml. plasma on days 7 and 8 of pregnancy.[4] Plasma levels in the present experiment need not, therefore, be considered unusual. On the other hand, 90 per cent absorption of a 5 mgm. pellet in 55 days means an average continuous absorption of 82 μg/day, seemingly not a small dose for a 25-30-gm. mouse. Increasing the pellet size from 5 to 10 mgm. caused a small but significant rise in plasma concentrations of progestin, but increasing the pellet size from 10 to 20 mgm. did not cause a significant further increase in plasma levels. This would seem to suggest that the mouse is able to enforce an upper limit of plasma concentration for exogenous progesterone. A similar phenomenon has been observed in women. Ober, Klein, and Weber[7] observed approximately the same plasma progestin levels in normal and castrate women after the intramuscular injection of oily solutions or microcrystalline suspensions of 100, 200, 300, and 400 mgm. of progesterone as after the injection of 50 mgm. There was only a modest further increase in the plasma concentration when 1,000 mgm. of the hormone were given.

SUMMARY

Each of 81 adult ovariectomized mice received subcutaneous implants of progesterone pellets weighing 5 to 20 mgm. After various time intervals the mice were exsanguinated, and the pellets were recovered and reweighed. They were 90 per cent absorbed in about 55 days. Plasma progestin levels as determined by bio-assay rose to between 3 and 4 Progesterone Equivalents/ml. within a few days, then declined, and thereafter

fluctuated irregularly. The high doses of hormone did not give consistently higher plasma progestin levels than did the lower doses.

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NEW COMPOUNDS WITH PROGESTATIONAL ACTIVITY: A REVIEW

INTRODUCTION

For some time steroid chemists have known that small chemical changes in steroid molecules may have profound effects on the biological activity of a compound. For example, a double bond inserted in the cortisone molecule greatly increases its biological activity as Δ-1, cortisone (meticorten). Likewise, removal of a methyl group from progesterone increases its biological activity, e.g., norprogesterone (Nor—signifying that something has been removed). In 1954 a group of investigators decided to try to increase the progestational activity of ethinyl testosterone (pregneninolone), a known potent oral progestogen, by removing this same methyl group. They succeeded in synthesizing this compound and a closely related one.[8] These compounds are known as the 19-nortestosterones (testosterone with a methyl group removed from the 19 position).

Early evaluation of these compounds disclosed that the predicted increase in progestational activity was present.[11] Since 1954 a considerable amount of pertinent information has been published concerning these products.

It is the purpose of this paper to review the literature on these preparations and to summarize the pharmacological and physiological findings to date. There are four compounds to be considered:

1. 17α methyl-19-nortestosterone (Neosteron-Organon).
2. 17α ethyl-19-nortestosterone (Nilevar-Searle).
3. 17α ethinyl-19-nortestosterone (Norlutin-Parke-Davis).
4. 17α ethinyl-5(10)-estraen-17β-01-3-one (Enovid-Searle). Fig. 1.

Progestational Activity

In addition to their obvious structural similarities, these compounds have several pharmacological properties in common. The foremost of these properties is their progestational activity. For the most part, these prep-

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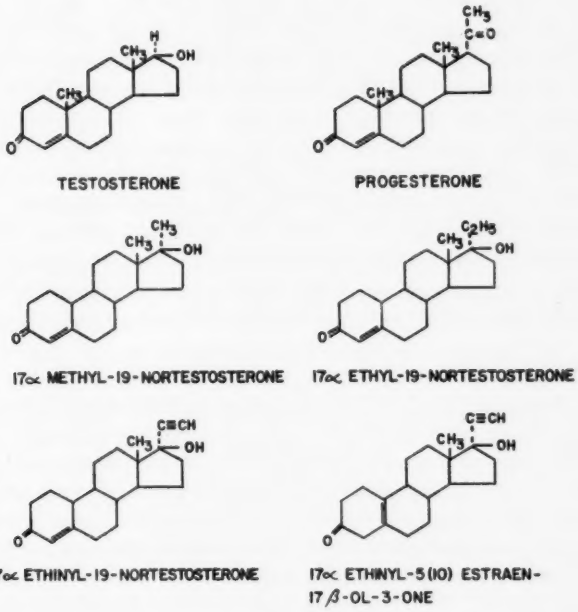
arations were evaluated quantitatively in estrogen-primed rabbits (Clauberg Test). All four were potent progestational compounds on this test showing from 2½ to 25 times the activity of progesterone per os.[11, 17, 20, 28] The compounds showed a greater activity than ethinyl testosterone.[11, 17]

FIGURE 1
STRUCTURAL RELATIONSHIPS OF TESTOSTERONE, PROGESTERONE AND FOUR COMPOUNDS WITH PROGESTIONAL ACTIVITY.



When the compounds were administered parenterally, there was decreased progestational activity. The compounds showed activity varying from less active to 10 times as active as progesterone.[20, 28] When administered directly into the uterus of the rabbit, the compounds showed less than one-fourth the activity of progesterone, probably indicating that some product of metabolism is the active hormone.[27, 28] Carbonic anhydrase studies in rabbit endometria, as a measure of progestational activity, showed rather unimpressive results, and mucus changes were atypical.[18] It is not possible to project the results of such animal experiments directly to man.

All four compounds have exhibited some degree of progestational activity in the human, the drugs showing a greater advantage over progesterone when administered by the sublingual and oral routes.[7, 9, 13, 32] One compound, 17α methyl-19-nortestosterone (Neosteron), shows 150 times the potency of progesterone and 50 times that of ethinyl testosterone when administered sublingually.[7] Parenterally, this great advantage over progesterone is lost, the effects ranging from 10 times the magnitude to less activity than progesterone administered by the same route.[7, 9] The drugs produce definite endometrial changes resembling those of progesterone.[7, 12, 23, 24, 33] However, Southam and Pincus found that in their patients there was a greater effect on stromal cells than glandular epithelium.[22, 30] Basal body temperature is elevated by administration,[9, 33] and there appear to be typical progestational cervical mucous changes.[7, 9] Withdrawal bleeding occurs with all four drugs in estrogen-primed women.[7, 23] The exact status of withdrawal bleeding caused by these compounds is rather confused since three of the compounds are known to be contaminated with estrogen.[20, 23, 24, 25] Alkaline phosphatase studies show changes more consistent with proliferative stages than with true secretory endometrium.[29] These studies would indicate that there is a distinct possibility of some variation from true progestational activity.

Prolonged administration of two of these compounds has demonstrated excellent decidual response in both human and monkey endometria.[19, 20, 23, 24, 32]

Androgenic Activity

Two of the compounds, 17α methyl-19-nortestosterone (Neosteron) and 17α ethyl-19-nortestosterone (Nilevar), as might be expected from their derivation, show androgenic activity.[3, 17, 25, 26, 28] In rats, this amounts to about 6 per cent of the activity of testosterone proprionate.[26, 27] One author failed to find any androgenicity in the rat.[8] It appears that these compounds have considerably less androgenic effect in humans than does testosterone.[25] Since esters of 19-nortestosterone exhibit quantitatively different androgenic activities,[1, 2, 6, 18] it is possible that as different esters of these compounds are investigated, similar changes will be demonstrated.

Anabolic Activity

The above-mentioned compounds, despite their rather poor androgenic activity, exhibit profound anabolic and myotrophic effects in animals.[6, 25, 26, 27] In studies on the *levator ani* muscle of rats, activity similar to testosterone propionate is noted.[26, 27] In the human, this anabolic and myotrophic effect is less easily demonstrated. Ferrin[7] showed no anabolic effect in humans with the methyl product (Neosteron), and Sachs[25] and Spencer[31] showed varying amounts of anabolic activity with the ethyl product (Nilevar).

Reproduction

There has been much interest in the effect these compounds have on the physiology of reproduction. They have multiple actions in preventing conception. All four compounds inhibit ovulation both in animals and humans.[20, 28] If ovulation does occur, there is some evidence that corpus luteum function is too poor to support a subsequent pregnancy.[28] This action may be due to either a direct effect on the ovary, or to secondary ovarian response to decreased gonadotrophin production. However, in most cases the ovulation response seems to precede and outweigh any measurable depression of the gonadotrophin titers. There is also some evidence that at least one of the compounds may have a damaging effect on any ovum that may be fertilized.[17] Heller,[10] in a controlled series of human experiments, has demonstrated that these compounds, as well as progesterone, cause complete azoospermia, although there may be some residual spermatogenesis in biopsy specimens. The compounds support pregnancy poorly. Pincus[20] claims that the ethyl compound (Nilevar) is a "potent maintainer of implantation." However, Saunders and Drill[27, 28] have shown that this compound is only 25 per cent as effective as progesterone in maintaining pregnancy. 17α ethinyl-5(10)-estraen-17β-01-3-one (Enovid) even seems to antagonize exogenous progesterone in its ability to maintain pregnancy.[20]

The decidual response shown by these drugs has been utilized by Rock *et al.* to produce a so-called pseudo-pregnancy and "rebound."[25, 24] The drugs were administered through two or more succeeding cycles with resultant amenorrhea and symptoms of early pregnancy. Rock noted that after cessation of therapy, a significant number of pregnancies occurred. Southam,[30] however, in a similar experiment demonstrated that the same degree of "rebound" could be obtained with progesterone and, in fact, the number of pregnancies after the "rebound" probably was not significantly higher than the number to be expected in an untreated series followed for a similar length of time.

Estrogenic Activity

All the compounds have some degree of inherent estrogenic activity besides the estrogen contamination mentioned earlier. The effect in the methyl (Neosteron) and ethyl (Nilevar) compounds is probably negligible,[17, 20, 25] the ethyl product (Nilevar) showing some anti-estrogenic activity.[4, 5] The ethinyl product (Norlutin) exhibits some definite estrogenic effects in rat experiments, and in humans probably causes some minimal estrogenic effect.[26, 27, 30] Jadrijevic *et al.*[11] have demonstrated some anti-estrogenic activity in the guinea pig. The estrogenic effects of 17α ethinyl-

5(10)-estraen-17β-01-3-one are high enough to be of significance in humans, and quantitation in animals shows it to have 1 to 7 per cent the activity of estrone sulfate.[20, 28] Since this estrogenic activity is even higher in the commercially available contaminated product (Enovid), the manufacturers have added estrogen to try to eliminate any break-through bleeding.

Effects on Gonadotrophin

In animals the compounds exhibit gonadotrophin-inhibiting activity.[9, 17] This inhibition is less definitely established in humans. The antigonadotrophic effect is not related to the androgenicity of the compounds.[8]

Metabolism

The intermediary metabolism of the four compounds is unknown. The knowledge of their effect on intermediary metabolism is equally sketchy. They show no effect on survival of adrenalectomized rats.[7] The ethyl compound (Nilevar) increases the serum α2 and β globulins and the β lipoproteins.[26] They produce relatively poor sodium retention.[14] The urinary pregnanediol levels are unaffected, as are the 17-ketosteroids and 17-OH-steroids, according to Greenblatt.[9] However, Pincus[22] claims a decrease of these same steroids. He also reports a decrease in blood progesterone levels and changes in the protein-binding of 17-OH-steroids. 19-nortestosterone has exhibited some very interesting adrenal protective reactions,[15] but thus far no such activity has been demonstrated for the progestational compounds.

Toxicity

Long-term administration of the compounds to both animals and humans shows no evidence of toxicity of permanent anatomical or physiological change.[17, 25, 31] However, Rock[24] does raise a question of damage to the primordial follicles. Side effects are not remarkable. Nausea, drowsiness, and anorexia are the principal untoward reactions,[9, 24] and occasional skin reactions have been reported.

SUMMARY

There are, therefore, four new synthetic compounds with the possibility of an infinite number of esters exhibiting progestational, myotrophic, and either androgenic or estrogenic activity (Table 1). At the present time it would seem that the compounds are probably more important from the standpoint of their chemical structure and the possibility that their small variations from the activity of progesterone may offer clues as to the func-

tion of various portions of the progesterone molecule. For the clinician, their advantage over the existing progestational compounds is less easily shown. 17*a* methyl-19-nortestosterone (Neosteron) would seem to offer a good progestational compound with minimal androgenicity. (The clinical studies on this compound have been done mainly in Europe and are less well known than on the other compounds). 17*a* ethyl-19-nortestos-

TABLE I

THE BIOLOGICAL ACTIVITY OF FOUR 19-NORTESTOSTERONES.

COMPOUND	PROGESTIONAL EFFECT	ANDROGENIC EFFECT	ESTROGENIC EFFECT	MYOTROPHIC EFFECT	INHIBITION OF GONADOTROPIN	CONTRACEPTION	SIDE EFFECTS
17∝ METHYL 19 NORTESTOSTERONE (NEOSTERON)	++++	++	−	+++	+	++++	+
17∝ ETHYL-19 NORTESTOSTERONE (NILEVAR)	+++	++	−	++++	++++	+++	++
17∝ ETHINYL-19 NORTESTOSTERONE (NORLUTIN)	++++	−	++	−	+	++++	+++
17∝ ETHINYL-5(10) ESTRAEN 17β-OL-3-ONE (ENOVID)	++++	−	++++	−	++++	++++	++++

terone (Nilevar) would seem to be the best anabolic agent of the four, with rather poor ability to prevent break-through bleeding and minimal androgenic activity. 17*a* ethinyl-19-nortestosterone (Norlutin) and 17*a* ethinyl-5(10)-estraen-17*β*-01-3-one (Enovid) demonstrate good progestational activity with minimal anabolic activity. However, both have definite estrogen effects. This has been compensated to some extent in Enovid by the addition of estrogen, so administration of the compound is essentially combined estrogen-progesterone therapy (Table 1).

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EVENTS

DEPARTMENT OF PSYCHIATRY SEMINAR

February 13, 1958

SENSORY DEPRIVATION. A NEW DEVELOPMENT IN PSYCHIATRY. By Philip Solomon, Physician-in-chief for Psychiatry, Boston City Hospital, Boston, Massachusetts.

Nine poliomyelitis patients treated in tank-type respirators developed psychotic-like symptoms after having been in the tanks for a few hours to a few days. Clinical evidence ruled out febrile, anoxic, toxic, and metabolic changes as the cause of these symptoms which consisted of well-organized visual, auditory, and somesthetic hallucinations and delusions.

To test the hypothesis that this temporary psychotic state was produced by the conditions of life in a tank respirator where the absolute intensity and meaningful pattern of stimuli were reduced, 17 normal male volunteers were confined in respirators for periods of time up to 36 hours with restrictions on vision, hearing, and touch. All the subjects experienced anxiety, oppression, inability to concentrate, and disorders in time judgment. Six subjects had pseudosomatic delusions, illusions, and hallucinations. The implications of these findings in clinical medicine, "brainwashing," and experimental production of psychosis by psychological techniques were discussed.

JOHN M. DAVIS

WOODWARD LECTURE

February 26, 1958

INFLUENZA, COLDS, AND SIMILAR ILLS. By Dr. Christopher Andrews, National Institute for Medical Research, London, England.

The recent pandemic of Asian influenza began in the interior of China early in 1957, and serological evidence indicates that the causative agent may be related to an influenza virus responsible for the pandemic of 1889-1890 which also arose in China; conceivably this area may have been the source of several other pandemics of influenza. The hypothesis is advanced that there is an animal reservoir in Central Asia and that periodically a new strain may arise from this source and spread into other parts of the world. The Asian virus belongs to the type A group of influenza viruses but is not closely related to any previously isolated strains. Except in the age groups above seventy, sera obtained from individuals before the pandemic of 1957 rarely contained any antibodies against the Asian strain of virus; this was thought to indicate a high degree of susceptibility of the population at large, and the subsequent world-wide spread of Asian influenza supported this idea. The presence of antibodies in the sera of older individuals suggested that a related strain of virus had been prevalent at about the time of the 1889-1890 pandemic. Studies on the mode of spread of

Asian influenza have revealed that, as in the case of most influenza epidemics, the spread is apparently facilitated by cold and damp weather. This may be related either to the degree of aggregation and crowding in the community, thus giving a better opportunity for the virus to spread, or it may be due to lowering of the hosts' resistance by local climatic conditions. In any event, the environmental and climatic factor in the spread of influenza seems to deserve more study.

With regard to the common cold, Dr. Andrews intimated that, from the standpoint of etiology, the term "common cold" is now outmoded, for evidence suggests that there are a variety of "colds" due to a variety of agents. These viruses continue to elude experimental study because of their apparent inability to propagate in any host except man and the chimpanzee. Practically, this confines experiments to human volunteers. In spite of this obvious limitation, much information has been obtained. The most reliable method of transmitting colds is by intranasal instillation of filtered nasal washings from subjects with colds. Interestingly enough, however, experimental transmission by close contact among volunteers is most unreliable.

J. R. PAUL

ZOOLOGY JOURNAL CLUB

April 9, 1958

FREEZING IN INTERTIDAL ANIMALS. By John Kanwisher, Woods Hole Oceanographic Institution.

Intertidal invertebrates and algae are able to live through the winter, even though they may be exposed to temperatures as low as -25° C. Within a few minutes after the tide recedes, the exposed organisms fall to the ambient temperature. Calorimetry on frozen animals indicates that as much as 80 per cent of the water present is frozen at -15° C. The remainder is bound water.

Histological examination revealed that all of the ice is formed extracellularly, causing the cells to become clumped, disoriented, and shrunken from dehydration. But immediately after thawing, the tissues appear normal again, and the animals are alive and active.

Respiratory Q_{10}'s were determined by gas analysis: Above O° C. the Q_{10} was a normal 2 to 3, whereas below freezing the Q_{10} ranged from 25 to 50. High internal osmotic pressures, produced by hypertonic medium or desiccation and corresponding in magnitude to those resulting from freezing, depress respiration sufficiently to account for the discrepancy between the Q_{10}'s of frozen and unfrozen organisms.

Closely related organisms in different geographical ranges differ widely in their resistance to freezing, according to the temperatures to which they are normally subjected.

F. M. W.

April 16, 1958

CORAL REEFS OF THE WEST INDIAN PROVINCE. By Norman D. Newell, Curator of Historical Geology and Fossil Invertebrates, American Museum of Natural History, New York, New York.

Reef corals in the West Indian province are represented by far fewer genera and species than the luxuriant Pacific group. Fossil evidence indicates that the two faunae have been discontinuous since the Miocene when the Mediterranean became separated from the Indian Ocean. Coral extended further to the north during the Cretaceous than in later periods. Oligocene forms in the Atlantic were far more diverse than today's, perhaps due to cooling and extinction but more probably caused by the Miocene separation.

Wide platforms ("reef flats") surround many Pacific atolls and islands probably as a result of planation since sea level reached its present height. Deep drilling has revealed that Tertiary reef limestone lies over volcanic rock, supporting Darwin's theory of continual growth during submergence. West Indian corals, however, form as encrustment on narrow submarine terraces which represent drowned shorelines. These drop off precipitously to thousands of feet. There are no broad reef flats as in the Pacific so that the islands are not protected; undercutting is common. Channels are found in noncoraline rock in the West Indies suggesting that similar formations in Pacific living coral are due to physical factors rather than coral growth patterns. Elevated reefs are found 10-15 feet above sea level. Coral types show conspicuous zonation. Branching corals extend down to 30 feet. A massive annularis zone follows. Below this edge, isolated corals stand on pedestals. At 65 feet, all coral growth stops.

The Bahamas lie on a 100-mile-wide sea level shelf extending north to Savannah, Georgia. Seismic studies indicate that this limestone shelf is 2½ miles deep. The shelf sequence, which began forming at the close of the Jurassie, is identical with that in the Florida Keys. Reef formation is restricted to the windward (eastern) sides of islands probably due to sedimentation, but isolated corals can grow on the leeward side without forming reefs. Hydrographic conditions determine the specific makeup of the community.

This evidence suggests the following geological history of West Indian reef formation: In the Mesozoic and Tertiary, a continuous barrier reef formed the continental shelf. This subsided slowly. The northern portion died during the Miocene, however, due to temperature and sedimentation. Corals survived south of Florida into the Pleistocene; no species were extinguished in this refugium. Profound changes in sea level due to glaciation and melting in this period allowed growth only on the highest parts of the shelf. Modern growth resumed only 3-4000 years ago.

G. E. W. 3RD

April 23, 1958

THE CHEMISTRY AND PHYSIOLOGY OF INSECT HORMONES. By Howard A. Schneiderman, Department of Zoology, Cornell University, Ithaca, New York.

A high level of juvenile hormone secreted by the corpora allata normally blocks metamorphosis in larvae of the Cecropia silkworm until the pupal stage from which the imago emerges. If a pupa is grafted to an adult male abdomen, however, the pupa metamorphoses into a second pupal stage showing some adult characters. As Carroll Williams first showed, a golden oil extracted from the male abdomen with a lipid solvent will produce the same inhibitory results when injected into a pupa. This juvenile hormone has now been partially purified. Various doses give quantitatively different intermediate results ranging from adult to almost pupal cuticle. The activity of the hormone can be bio-assayed on various pupal organs. Antennae show the most sensitive assay. Regenerating tissue is also especially sensitive to hormone administration. A Polyphemus unit is defined as that amount producing a detectable change in 7 of 10 pupae. The hormone must be administered when the pupa is on the brink of developmental change (i.e., metamorphosis) or it will be broken down. A five-gamma dose of concentrated extract of juvenile hormone causes a noticeable change.

The purified hormone is remarkably stable; it is not inactivated by heating for four hours at 140° C, nor by hydrogenation nor mild saponification. The purified extract inhibits complete adult metamorphosis of almost all insects if administered at the right stage.

Juvenile hormone has been extracted from adults of 30 species of Lepidoptera in far greater concentration than from the larval instars; also from unfertilized eggs and diapausing pupae; from beetles; and from beef adrenal cortex. This beef extract mimics in detail the action of the insect hormone. It is rich in steroid material which may be the active principle. This finding suggests that the juvenile hormone itself may be a steroid and that steroid hormones, rather than being a recent innovation of the vertebrates, have a far more ancient lineage.

G. E. W. 3RD

April 30, 1958

TRANSPLANTATION OF ANTIBODY-PRODUCING CELLS. By N. Avrion Mitchison, University of Edinburgh, Scotland.

Inbred mouse strains may be used as immunological "test tubes" since tissue culture cells do not retain their normal antibody function after division *in vitro*. Transplantation experiments have provided information on antibody-producing cell autonomy, localization, multiplication, and reactivity.

Adoptive immunity may be induced in a host mouse by implanting lymph nodes from a mouse of the same strain previously immunized to implanted tumors from a mouse of a different strain. Tumor implants are reacted against far more vigorously than in controls. Such adoptive immunity

may be demonstrated in host mice rendered tolerant to the challenger strain. This evidence indicates that antibodies are produced autonomously by the donor lymphoid implant. Mechanically disaggregated spleen cells from a mouse immunized against *Salmonella typhi* actively produce antibodies when injected intraperitoneally. Controls receiving only antiserum or killed spleen cells show passive immunity which is progressively lost. Antibody titers in the experimental animals are maximal at five to seven days after injection. They remain high in isologous hosts, but in homologous hosts, decline with about the same half-life as in controls. Thus, in the homologous hosts, implanted cells are killed by host antibodies after one week. In a homologous host which has been previously immunized, the implanted tissue will be killed even sooner. This experiment further confirms the existence of autonomous antibody production by the host implant cells.

Lymph node cells from an immune mouse transferred subcutaneously to a host also produce antibodies autonomously. If the cell nodule is removed after four days, antibody production is impaired, but after ten to twelve days no impairment takes place following removal. Therefore, functionally active donor cells have emigrated from the subcutaneous site.

After host antibody-producing cells are x-irradiated with 500r, antibody titer increases ten-fold when donor spleen is introduced. This suggests that donor cells capable of producing antibodies multiply with genetic continuity. This view is further supported by "cellular memory" experiments. An organism which has stopped previously induced production of antibodies will show higher antibody levels when challenged a second time than controls not previously immunized.

Intravenously injected adult turkey erythrocytes are tolerated by chick embryos but agglutinate in embryonic blood vessels. If leucocytes are thus introduced, however, donor immunological reactions set in as side effects similar to "runt disease" in mice. An enlarged spleen is one symptom of this reaction in pseudochimaerae. This enlargement is probably due to antibodies from the donor lymphoid tissue coating the chick cells; it may also be due in part to colonization by donor cells which apparently become localized in the homologous tissue of the host.

Experiments of this sort have clinical applications in transplantation of human tissues.

G. E. W. 3RD

YALE MEDICAL SOCIETY

March 10, 1958

DUAL INFECTION OF CELLS WITH MEASLES AND POLIO VIRUS. By Magdalena Reissig, Research Associate in Epidemiology and Preventive Medicine, Francis L. Black, Research Associate in Epidemiology and Preventive Medicine and Lecturer in Microbiology, and Joseph L. Melnick, formerly Professor of Epidemiology at Yale University School of Medicine.

As shown by Anderson (1942), and others, two viruses which cause the production in the host of the same intranuclear or cytoplasmic inclusions

are mutually exclusive, but if there is a differential localization of inclusions, double infection is possible. In the present experiments, dual infection of monkey kidney cells in culture was effected and analyzed morphologically.

In the normal kidney cell, the nuclear membrane is indistinct, but a chromatin network is visible. Cells infected by poliomyelitis virus show a more distinct nuclear membrane which gradually becomes considerably wrinkled, and a chromatin network which slowly disappears as eosinophilic inclusions appear in the nucleus. Somewhat later an eosinophilic paranuclear mass is prominent in the cytoplasm of these normal-sized rounded cells. Contrasted with this, measles virus characteristically produces giant cells with a homogeneous eosinophilic cytoplasm, intranuclear inclusions of a larger size than in polio, and causes no wrinkling of the nuclear membrane. Each of the inclusions is surrounded by a "halo" of light-staining chromatin and is itself less eosinophilic than a polio inclusion.

Since the polio virus has a shorter cycle than that of measles, cells were first infected with measles and 4-5 days later with polio. These doubly infected cells, when examined histologically after fixation with Zenker-formol, at once resembled measles—and polio-infected cells, some having an eosinophilic cytoplasmic mass and small inclusions while also containing halo-surrounded inclusions. Polynucleate cells had normal and abnormal nuclei within one plasma membrane. In one instance, five shrunken nuclei, an eosinophilic mass and large intranuclear inclusions were encountered in a single cell. Virus assays carried out at 20, 48, and 72 hours after secondary infection by Type 1 polio virus indicate that polio virus yield in the doubly infected cells is as high as in mono-infected cells. Objections that the 10 per cent of the cells uninfected by measles in the 20-hour group contributed disproportionately to the total yield were overcome by examination of single cells. These were isolated under oil after having been inoculated with polio virus five days after measles infection, incubated for 1½ hours, washed, and suspended in versene. After seven hours at 37° C. to allow for virus particle formation, assays were run and compared with controls and mono-infected material. Giant round cells had as many particles as singly infected cells, some having more than 1,000 particles. Giant flat cells (dead) of equal surface area had 0-20 particles, ruling out adsorption effects. The site of production and precise nature of polio inclusions are unknown, although it is certain they are neither DNA nor RNA. That they are neither the virus nor precursors is proved by their appearance after the completion of virus production in the host.

Morphological evidence thus suggests that double infection is possible, producing intranuclear and cytoplasmic inclusions, at least for measles and poliomyelitis virus, and that the doubly infected cells produce polio virus comparable to that of polio-infected cells alone. Clinical observation of this phenomenon has not been made, since the epidemic seasons for measles and polio differ.

MALCOLM S. MITCHELL

April 14, 1958

A New Look at Pulmonary Pathology. By Jethro Gough, Professor of Pathology, Welsh National School of Medicine, Cardiff, Wales.

The preparation of sections of whole lungs is a technique which facilitates correlation between roentgenological studies and pathological anatomy. Sections are generally cut sagittally, rather than coronally, in order to emphasize the summative aspect of the x-ray shadow. One aspect of lung pathology thus emphasized is that of the lobule. Lobular septa penetrate into the lung and are recognized roentgenographically as Kerley's "B" lines, as for instance when edematous in mitral stenosis. In "honey-comb" lung, new formed fibrous tissue, which is radio-opaque, outlines the lobule, and many pathological varieties of this pattern may be distinguished from each other.

More than half of the cases of large lung emphysema examined show a centrilobular lesion due to destruction of tissue and dilatation of respiratory bronchioles. In uncomplicated asthma, even of long standing, this lesion is never seen.

It is emphasized that this technique furnishes a superb pedagogical tool, applicable not only to lung, but also to bony and porous tissues. Sections are mounted on paper, and it is possible to build up rather extensive tissue libraries with truly minimal demands on space.

N. R. M.

NU SIGMA NU LECTURE

April 17, 1958

Historical Considerations in the Treatment of Burns. By Carl A. Moyer, Professor of Surgery, Washington University, St. Louis, Missouri.

There has been a variety of methods of treating body burns over the years including the use of grease, Syme's dry cotton wool binding, carbolized oil, picric acid, aluminum acetate, and tannic acid application, and a host of others. Interestingly enough, the over-all mortality from burns rose from about 17 per cent in 1833 to a peak of 38 per cent in 1903, after which the figures began to decline. The mortality from 25 to 100 per cent body burns in 1903 was 100 per cent, but in 1905 Sneve in St. Paul introduced the "open dressing" method and early grafting along with the administration of saline orally, per rectum, and subcutaneously. In that year the mortality in this 25 to 100 per cent burn group declined to 50 per cent, and even today the figure has only been reduced to 30 per cent.

At this point we have more information available to us. Underhill showed that there is a body loss of water and sodium when burns occur. In addition, it has been found that a decrease in body sodium leads to decreased oxygen utilization and shock, hence the rationale for saline administration. Lesser demonstrated that the red blood cell mass is decreased immediately after a burn. Later evidence showed that heat leads to stasis of flow in capillaries as well as hemolysis of red blood cells. One stumbling block in the past was that people believed that a high hematocrit, due to

hemoconcentration in the case of burns, was incompatible with life, but Moyer and others showed that burn patients could be given whole blood with safety. Thus it seems that in moderate shock saline, in preference to plasma expanders, should be used; and in severe states whole blood should be added to this.

Newer experimental evidence gives us further information. Basal metabolic rates are increased when burns are left open, and this is due to an increased rate of water vaporization and of heat loss. Dressings and early grafting prevent water from going off, lower the BMR to nearly normal, and now we also know that they help to prevent infection. Diet used to be a worry, too, but the author and his coworkers have shown that rats losing 40 per cent of their body weight on a protein-free diet for 80 days healed 20 per cent burns just as quickly as did control rats.

Finally, if we consider the results of treatment, most of the progress has been made in the 25 to 65 per cent burn groups with a mortality of 26 per cent or less. Almost no therapy in the less than 20 per cent burn is as good as anything. The results of treatment of greater than 65 per cent burns have not been appreciably improved. One method of approach would be to prevent the occurrence of burns by improving home engineering and making clothing from nonflammable fabrics such as wool, silk, or pure synthetics. The equivalent of six one-thousand-bed hospital units is required yearly to care for burns, so it would behoove us to think more along these lines.

J. A. O'N.

THIRD ANNUAL LEON E. SAMPLE LECTURE

May 7, 1958

PANCREATITIS. By Charles G. Child, III, Professor of Surgery, Tufts University School of Medicine, Boston, Massachusetts.

Pancreatitis is commonly associated with disease of the biliary tract, particularly cholelithiasis. However, as opposed to the usual high incidence of gall bladder disease in obese women, pancreatitis is usually found in thin men over 40 years of age. Other dissimilarities between these entities may be seen in the role of surgery and the use of morphine during the acute attacks, both employed in gall bladder disease but contra-indicated in pancreatitis. Another important correlation is the high incidence of pancreatitis among alcoholics, many of whom are under 40 years of age. Two rare types of pancreatitis have been described, one being a Mendelian inherited trait starting in childhood, the other being found in certain forms of hyperlipemia; neither of these entities has contributed much to our basic knowledge of pancreatitis.

The etiology of pancreatitis is not well understood. Gross and microscopic studies as well as animal and biochemical experimentation have contributed but incompletely to this understanding. The common channel theory proposed that with obstruction at the ampulla of Vater (e.g., by a gallstone) a common channel exists whereby bile flows from the common duct into the pancreatic duct to produce acute pancreatitis. Since its intro-

duction in 1901 by Opie, there have been conflicting reports in support of this theory. More recently, the experimental creation of a common channel without the production of pancreatitis in the goat, the failure to demonstrate a common channel with IV cholangiograms, and physiological studies which indicate that the pressure in the pancreatic duct is equal to or greater than that in the common duct all have tended to discount the validity of the common channel theory. On the other hand, present evidence supports the view that partial obstruction of the pancreatic duct in association with maximal stimulation of pancreatic exocrine secretion is operative in the production of both acute and chronic pancreatitis. The factors which may be responsible for producing partial obstruction of the pancreatic duct include edema, cicatrization, and duodenal wall compression. The latter may explain the failure of sphincterotomy in certain cases.

S. I. H.

PRESENTATION OF THESES OF CLASS OF 1958

May 22, 1958

THE INACTIVATION OF SECRETIN. By J. W. Love.

Greengard, Stein, and Ivy reported in 1941 that secretin is inactivated by serum when the two are incubated together *in vitro*. The inactivation was observed to proceed optimally at 37° C. and at neutral pH with a fall-off in the speed of inactivation as these parameters were varied above or below the optimal values. The degree of inactivation varied directly with the concentration of serum, and serum previously heated at 60° C. for 30 minutes was ineffective for the inactivation. From these observations it was concluded that serum contains an enzyme which inactivates the hormone; Greengard, Stein and Ivy proposed the name secretinase for this substance.

The project began as an attempt to repeat the work of Greengard, Stein, and Ivy using hog secretin obtained from Oxford University, rat serum, and the rat assay for secretin described by Love. Early experiments established that rat serum does inactivate hog secretin when the two are incubated *in vitro,* that serum previously heated at 37° C. for 30 minutes is ineffective for the inactivation, and that the inactivation proceeds optimally at 37° C. This much stood in confirmation of Greengard, Stein, and Ivy. While investigating the effect of varying serum concentration in the system, it was observed that the sera were only weakly effective for inactivating the hormone; the undiluted serum from one rat was totally inactive against secretin. The experimental protocols were reviewed in an attempt to discover some alteration in the experimental conditions which might have accounted for the apparent loss of secretin-inactivating capacity that was being observed.

Only the technique for obtaining serum for incubation purposes had changed. The technique consisted in anesthetizing a rat with ether, and, when the animal was sufficiently deep, opening the anterior chest to obtain blood by direct cardiac puncture. In the earlier experiments the anesthet-

ization of the rats to be exsanguinated was prolonged and awkward due to the inexperience of the author; with practice the anesthetization was accomplished quickly and smoothly with a minimal phase of excitement. To test the possibility that the technique of anesthesia might be related to the secretin-inactivating capacity of the serum, two subsequent rats for exsanguination were anesthetized with deliberate slowness to encourage a long phase of excitement. Significant inactivation of the secretin solution was produced with both sera. The thought occurred that adrenal-cortical activity might be playing a role; the cortices of an exsanguinated rat were extracted with saline and the clear filtrate added to secretin. The astonishing result was an 86 per cent inactivation after three minutes of incubation, total inactivation of the hormone after two hours of incubation. This effect of adrenal cortical extract was verified in subsequent experiments. Extracts of adrenal medulla and skeletal muscle did not appear to inactivate the hormone. No apparent inactivation of the hormone was produced by incubations with cortisone, hydrocortisone, or prednisolone.

Conclusions drawn from these preliminary observations are that secretin derived from hogs can be inactivated by rat serum *in vitro* at neutral pH and 37° C., but the secretin-inactivating capacity of rat serum is labile and some sera are totally inactive against the hormone; that a saline extract of rat adrenal cortex can regularly, rapidly, and completely inactivate secretin at 37° C.; that the adrenal cortex may produce one or more factors which are responsible for inactivating, or initiating the inactivation of secretin.

THE MECHANISM OF HYPOCHLOREMIA IN RESPIRATORY ACIDOSIS. By William C. Branscome.

It has been repeatedly observed that, in animals and man, a decrease in the concentration of plasma chloride and a reciprocal increase in the concentration of plasma bicarbonate characteristically develops following exposure to an increased concentration of inspired carbon dioxide. Acute studies of CO_2 inhalation have heretofore failed to demonstrate losses of chloride in the urine in excess of sodium. It has therefore been suggested by some that the hypochloremia of respiratory acidosis might arise from the transfer of chloride to an intracellular position, particularly a shift into red cells, or from the dilution of the extracellular fluid with sodium bicarbonate. This study was undertaken in an effort to determine whether increased urinary excretion of chloride might continue to the hypochloremia of respiration acidosis.

Twenty-four male albino rats with average weights of 375 grams were placed in individual metabolism cages and were given Purina lab chow and water *ad lib.* Urine was collected under mineral oil, feces being excluded but not analyzed. The experimental groups were placed in an airtight plastic chamber through which was circulated a mixture of 7½ per cent CO_2 in air or 12 per cent in air for periods of one to four days. All animals were anesthetized with Nembutal immediately following removal from the chamber, the entire exsanguination procedure requiring from four to six minutes.

On exposure to 7½ per cent CO_2 serum changes typical of respiratory acidosis were produced, with an elevation of the serum bicarbonate of about 10 mEq./L and a reciprocal and equal depression of serum chloride. The concentration of chloride in red blood cells increased 9 mEq./L, but this shift accounted for only 15 per cent of the chloride lost from the extracellular space. No significant change in extracellular fluid volume was produced. Potassium was lost from the intracellular fluid space but no significant change in the quantity of sodium in the intracellular fluid was observed. During the first 24 hours of exposure, a significant negative balance of chloride was observed, accompanied by an increased renal excretion of potassium and ammonia and a decreased urinary pH. These balances returned to normal by the second day.

A decreased intake of food was noticed in those rats exposed to 12 per cent CO_2. The serum changes were similar to those seen in the previous group, with an elevation of the serum bicarbonate of about 15 mEq./L and a depression of the serum chloride of about 15 mEq./L. The chloride shift into red cells accounted for less than 2 per cent of the chloride lost from the extracellular fluid. Extracellular fluid volume decreased 14 per cent, and potassium was lost from the intracellular space. The first day of exposure was marked by a net output of chloride in excess of sodium, a negative balance of potassium, and an almost threefold increase over the normal in the excretion of ammonia. The balances returned to normal by the third day, though ammonia excretion remained high.

Exposure to room air following two days of a 12 per cent CO_2 atmosphere produced a return of the electrolyte pattern to normal. There was a significant positive balance of chloride, potassium, and sodium on the first day of exposure to room air, accompanied by a decreased ammonia formation and an increased urinary pH.

These data suggest that renal compensation, characterized by excretion of chloride in association with potassium and ammonium, plays an important role in adjustment to respiratory acidosis in the rat. It may be that there is a tubular mechanism for anion absorption and anion exchange that operates independently of the mechanisms already described for the exchange of cations. If this were the case, inhibition of active chloride reabsorption by CO_2 would explain the findings encountered in the present study.

BOOK REVIEWS

ENDOCRINE PATHOLOGY OF THE OVARY. By John McL. Morris and Robert E. Scully. St. Louis, C. V. Mosby Co., 1958. 151 pp. $8.50.

This monograph attempts to make relative order out of relative chaos in a field where the limitations of our present knowledge are severe and restrictive. The attempt is successful for the authors have exhibited a fine sense of discrimination between what is known, what is tenable, and what is conjectural. In addition they manifest a rapport between clinician and pathologist which is as uncommon as it is necessary if advances in knowledge are to be made.

The plan of the monograph is conventional and straightforward. The opening chapter is anatomical, dealing with the cells of the gonads, their embryology, matters of sex differentiation, and sex hormone production. Here as well as elsewhere they emphasize the ambivalence of end-organ responses to specific hormones and the incompleteness of our current concepts of "estrogen" and "androgen" as well as "feminization" and "masculinization." The fundamental anatomical information is presented in a critical fashion and leads directly to a discussion of hormone assays, their methods and limitations, followed by a classification of endocrine effects as manifested clinically.

With the above background established, there is a discussion of non-neoplastic abnormalities of the ovary with endocrine effect. This includes such topics as the polycystic ovary-hyperthecosis-Stein-Leventhal syndrome and the concept of cortical stromal hyperplasia. For the first time in the reviewer's experience these two confusing matters are defined, illustrated, and elaborated in a manner which makes sense clinically and pathologically.

The largest portion of the monograph is devoted to a classification of "functional" ovarian tumors and a discussion of the features which are associated with each variety. The basis of the classification is morphological; the authors point out that endocrine effects cannot be used as a basis for systematic taxonomy in view of the fact that similar effects can be ascribed to a variety of cell types, and that absence of demonstrable endocrine effects does not exclude the endocrine cell origin of a tumor. Their classification is simple but comprehensive; it is as easy to follow in practice as it is sound in theory. The ensuing chapters which discuss the clinical, endocrinological, and pathological characteristics of the specific tumors are scholarly, due attention being given to the necessary qualification of general statements.

One of the most useful features of the monograph is the careful selection of illustrations. There are 75 black and white photomicrographs; none is less than good and many are superb. Any pathologist who has attempted to illustrate the critical morphological features of this type of neoplasm will appreciate the judgment used in the selection of fields as well as the care in the preparation of the material. No important feature has been left unillustrated. One might wish for a list of illustrations following the table of contents, but this would rob one of the aesthetic pleasure of leafing through the book and seeing the other pictures as one searches for a specific item. The authors are to be complimented for having resisted the temptation to include a colored plate showing a gross specimen of a spherical

or ovoid mass with a yellow cut surface; in the reviewer's experience the fact that a solid ovarian tumor has a yellow tint on section is of no diagnostic value at all; this feature characterizes many of the members of the group of "functional" tumors (except those that are various shades of grey), and the price of the book would have been higher had they succumbed to temptation.

The text is well documented by a carefully selected bibliography which includes the titles of articles as well as the reference. There is a fair sprinkling of references to articles of historical importance, and there is no hesitation in utilizing the British, French, or German literature when relevant. It is refreshing to learn that medicine is international; so many recent articles in this field have utilized only American sources of reference that the reviewer was becoming suspicious of a cartel.

However, the above are only ancillary virtues which reflect the intellectual distinction of the authors. The great value of the monograph is its ability to correlate systematically in a concise and lucid style the available information regarding this group of tumors. Yet the authors have not been content merely to summarize. They indicate clearly what is unknown, and each lacuna in our knowledge should serve as a stimulus for further study.

The text is authoritative and readable. It supersedes previous books devoted specifically to ovarian tumors as well as those chapters in more general texts which deal with the subject.

WILLIAM B. OBER

RECENT ADVANCES IN UROLOGY. By Howard G. Hanley. Boston, Little, Brown and Company, 1957. 270 pp. $7.00.

This publication is an interesting and valuable adjunct to a urologist's library. There are fourteen chapters, each dealing with a separate aspect of urology and urological surgery. The first chapter, dealing with renal function, reduces the complexity of this topic to the fundamentals of physiology. It is both informative and interesting reading. The chapter dealing with prostatic resection is flavored by preferences for retropubic approaches, but this does not detract from its excellent coverage. The chapter on radiology touches on cinefluoroscopy as a diagnostic tool, but perhaps not enough emphasis is given to the potentialities of this technique. Since this book has been published, interpretation of urological cinefluoroscopy has been accepted by most centers. It is agreed that there is much to be learned in the interpretation of cinefluoroscopy, but many important advances in the near future may be made through this medium.

The coverage of urinary diversion is excellent in reference to technique and metabolic derangement from these procedures. The author carries urinary diversion through its chronological and logical development through the current use of ileal segments.

I am somewhat surprised that there is so little mention of renal homo transplantation as carried out by the Boston group. This probably is one of the most outstanding advances in the recent past. I am sure that this is not due to omission but rather that the publication was dated prior to

this work. Intracavitary photography has not been covered, either, and would have been of current interest.

I think it is extremely unusual for any book to include so much in such a concise and clear manner as has been accomplished by Dr. Hanley. The bibliographies are excellent and condensed to the important contributors.

ARNOLD M. BASKIN

MANAGEMENT OF COMPLICATIONS IN EYE SURGERY. R. M. Fasanella, Ed., Philadelphia, W. B. Saunders Company, 1957. xv, 422 pp. $16.00.

In this work, twenty-three contributors, mostly from the field of ophthalmology, express their opinions on the factors leading to complications in eye surgery, their avoidance, and their management. When one considers the many recent advances in this subject and the necessary exactitude of the techniques, the value of such a book becomes apparent.

Chapters devoted to general medical and psychiatric complications deal with the selection of patients, the evaluation of the surgical risk and the preoperative, operative, and postoperative care. The topics of anesthesia, medical and radiation ocular therapy in the avoidance and treatment of complications, and the rehabilitation of the low vision patient contain many helpful points which may mean the difference between success and failure in any given case. Specific complications of a wide variety of operations ranging from the frequently performed procedures for glaucoma control and cataract extraction to the more rarely performed operations such as orbital decompression for thyrotropic exophthalmos are written in a highly personal style by men well experienced in each field. Only those procedures which are currently popular, and in certain chapters only those which are favored by the particular author, are included. The book is well up to date, including such subjects as surgery of the vitreous, keratoplasty, and the new mobility implants, as well as many recent references.

In places the book falls short of its over-all high calibre due to incomplete coverage or hasty skimming of certain topics as well as some authors' failure to include any bibliography. The editor's organization of the material is good and the publishers are to be congratulated on the excellent printing and photographic reproductions. This work is strongly recommended both for general reading and as a reference text for anyone in the field of ophthalmic surgery.

T. W. LIEBERMAN

PREVENTIVE MEDICINE FOR THE DOCTOR IN HIS COMMUNITY. By H. R. Leavell and E. Gurney Clark. 2nd Ed., New York, McGraw-Hill Book Company, 1958. 689 pp. $10.

The second edition of Leavell and Clark's Textbook of Preventive Medicine, which follows the first by five years, reflects changes which have occurred in that time. The authors have turned their attention more to clinicians as is evident from the change in the title of the book from the original which mentioned preventive medicine alone. In addition to the change in title they have added a subtitle, "An Epidemiologic Approach." The book has also increased slightly in size, and understandably, in price.

It is clear that the authors have tended more and more to express in this book their fundamental belief that any physician who sets out to prevent disease will be better equipped to do so if he is grounded in epidemiologic principles. A familiar statement one hears today, usually from clinicians, is that the unqualified term, "preventive medicine" is outdated, as far as they are concerned. Pediatricians are now equipped and trained to take care of the preventive aspects of their profession; obstetricians in like manner know best how to take care of maternal and child welfare. Psychiatrists lead the way in mental hygiene, and the internist is certainly regarded as inadequate, if, in the care of his patients, he is concerned only with "curative" medicine. Indeed, it is difficult for the modern clinician to distinguish between preventive and curative medicine. Why, then, one may ask, or they may ask, should there be any attempt to make or to individualize *preventive medicine* as opposed to curative medicine, particularly as the former is a rather boastful kind of a term.

Some of the answers to these questions will be found in this book, for it has been a very special concern of the authors to explain this very point. In the 40 pages which Dr. Clark devotes to the chapter entitled, "An Epidemiologic Approach to Preventive Medicine," he goes to some length to point out that the pediatrician, internist, or obstetrician who believes himself well equipped to take care of the preventive aspects of his work, would probably be better qualified to do this if he applied epidemiological reasoning to his clinical skills. This point of view is also an answer to those who have maintained that there is no need for "departments" of preventive medicine in modern medical schools.

The authors present in various chapters a number of features on which the average clinician, particularly if he has been out of medical school for a dozen years, may have had limited training. First among these, as already mentioned, is a grounding in epidemiological thinking. Second, is training in biostatistics, a subject for which all would-be epidemiologists should have increasing respect. Another chapter concerns population problems, long-term illness as a community problem, and the effect of the aging process on health. Still another deals with genetics for the clinician.

Considerations for the public health officer, whose responsibilities are concerned with preventive medicine and methods of organizing a community for action in this respect, also receive a fair amount of attention in the last 160 pages. However, the main purpose of this volume is directed towards the physician so that he may be informed that there is more to be learned about the *modern* care of patients than can usually be found in the latest editions of most of the modern *clinical* textbooks.

JOHN R. PAUL

IN SEARCH OF MAN. By André Missenard. New York, Hawthorn Books, Inc., 1957. 346 pp. $5.95.

How can one review an encyclopedia? This book runs the gamut from acclimatization to zygote. In between is collected a considerable mass of information ranging from genetics and heredity to the spiritual and moral aspects of mankind. Included are considerations of the effect of the chem-

ical environment on human activity and behavior, as well as the physical and climatic milieu.

Unfortunately, the book is slanted. Since the author worked with Carrel, the writing is colored by the dualistic approach to the nature of man. While admitting that man is a physical chemical machine, his activity is governed in part not only by the physical laws of the universe but, also, by spiritual and moral laws. The author pleads for the development of an élite through the application of what we know about genetics, nutrition, climate, and education. Inherent in the argument is the belief that the social organizations of man are more important than the individual. This is always the argument of the moralist who thinks he knows all the answers and would make others conform to what he believes is right.

The author proposes the development of better individuals but only because they can thereby build better communities. He admits that the whole is greater than the sum of its parts, but forgets that the whole cannot exist without adequate components. Very clearly he assumes the existence of a moral law which is something over and above the laws of the material universe. It will be remembered that Sir Charles Sherrington in his important book, *Man on His Nature,* calls attention to the difficulty of explaining how mind, which exists in neither time nor place, and which involves no energy transformations, influences the brain which does exist in space and in time, and which does involve energy transformation.

This is the key problem, of course. In all studies of man one must decide, largely as a matter of belief, whether or not man is a special creation endowed with a mind or a spirit which transcends the physical, or that man is a collection of material substances subject to natural law which, because of incredible complexity, acquire qualitative attributes that the dualists prefer to call mind, or soul. For the dualist, this mind or soul recognizes the existence of moral law which transcends natural law. Unfortunately, this denies the unity of the universe. Moral law then becomes a matter of belief which cannot be subjected to the same kind of verifiable analysis to be found everywhere in the physical universe.

It would seem much more intelligent to hold that what is true is right, and what is wrong is evil. Since our understanding of what is true changes as our knowledge and wisdom grow, so moral law changes accordingly. None of us knows all the answers; how stupid the world would be if we did. It is dangerous to assume, therefore, that the moral law for any generation holds throughout all generations. This does not deny the existence of absolutes, but only asserts that man is struggling toward them, but has not yet reached them.

If you are a dualist, this is an interesting and informative book; if you are not, it is maddening.

H. S. BURR

ATOMIC ENERGY IN MEDICINE. By K. E. Halnan. New York, Philosophical Library, Inc., and printed in Great Britain for Philosophical Library by Industrial Press, Ltd., South-end-on-Sea, Essex, 1957. 157 pp. $6.00.

This book was written by a British radiologist for readers not necessarily having much knowledge of either physics or medicine. Although it is re-

garded as a general introduction to the interesting new field of atomic medicine, it covers a vast amount of information on radioisotopes which are used in medical research, diagnosis, and therapy. The background of nuclear physics on each aspect of this new field of medicine is concisely explained.

The medical use of high energy particle accelerators such as cyclotron, betatron, synchrotron, and linear accelerators are briefly described. The linear accelerator is considered as the machine which has real practical value in radiotherapy. In radiotherapy today in England there is considerable competition between linear accelerator and large telecurie units, such as Cobalt-60 or Cesium-137. Both these developments are important by-products of atomic energy development.

The three dangerous biological effects of radiation to man are discussed in some detail: (1) immediate danger of tissue damage from individual high exposure, (2) long-term danger of chronic damage to tissue with possible induction of cancer, (3) remote but important danger of permanent genetic damage that may seriously affect future generations.

The common levels of radiation exposure are listed in detail based on the data published in Great Britain. Some interesting estimated figures on radiation received by the whole population of Great Britain to reproductive organs in the first 30 years of life as a genetic hazard are abstracted as follows: Natural body radioactivity, 690 mr. (milliroentgen); cosmic rays and terrestrial radioactivity, 1770 mr.; diagnostic x-ray to patients, 630 mr.; luminous watches, 30 mr.; atomic bomb explosions, 26 mr.; shoe-fitting machines, 3 mr.; air crew from luminous aircraft instruments, 0.3 mr.; occupational, 50 mr. The dose required to double human mutation rate is estimated to be 50,000 mr. The author also stresses the opinion that genetic damage by radiation is not yet fully understood and must be worked out as one of our most urgent problems.

The material chosen is mainly from the British sources and is based on a comprehensive review of the literature of the subject. The illustrations are excellent but no bibliography is appended. It is easy and pleasing to read and it should be recommended to medical students, nurses, and physicians who want to have a general and up-to-date view of this subject.

C. H. CHANG

THE STRATEGY OF THE GENES. By C. H. Waddington, with an appendix by H. Kacser. New York, Macmillan, 1958. ix, 262 pp. $4.00.

"How does development produce entities which have Form, in the sense of integration or wholeness; how does evolution bring into being organisms which have Ends, in the sense of goal-seeking or directiveness?" An examination of these questions provides the unifying theme for this collection of essays which are Professor Waddington's most recent contribution to theoretical biology. Although portions of four of the five essays have appeared elsewhere in different form, they are essentially new and contain much unpublished experimental work in support of theoretical arguments. There is an appendix entitled "Some Physico-Chemical Aspects of Biological Organization," which has been contributed by Dr. H. Kacser of the Institute of Animal Genetics in Edinburgh. Dr. Kacser discusses sev-

eral kinetic systems and compares their properties (after cautioning the reader of the possible pitfalls and of the assumptions which must be made) with various biological phenomena such as regulation, regeneration, determination, gene action, pleiotropism, etc.

Few biologists have been as successful as Waddington in accomplishing a synthesis between embryology and genetics. In this volume, he brings his wide knowledge of these two fields into the framework of modern evolutionary thought in a concerted attempt to develop a theory of evolution which takes advantage of the concepts of developmental genetics as well as those of modern population genetics.

A glance at the titles of the essays in the collection—Form, End and Time; Selection Of, For and By; The Organization of the Gene Pool; and The Survival of the Adaptable—is sufficient to indicate that some of the thorniest problems in modern evolutionary thought have been approached. (One of the essays, The Cybernetics of Development, is concerned largely with embryological problems, especially the canalization of development.) Waddington has been scrupulously careful to define terms and concepts as he uses them, and one of the great merits of the book is that it avoids much of the muddled thinking and careless use of terminology so common in present-day literature.

He examines the contributions to modern evolutionary theory which have been made by the mathematical approach and concludes that, in spite of much enthusiasm for the method and the rather awe-struck attitude toward it of nonmathematical biologists, it has contributed very little to evolutionary thought at the theoretical level. He makes this point repeatedly " . . . in order to overcome the anaesthetic effect exerted on biologists by algebra which is incomprehensible to them. There is no need for evolutionary theory to limit its view to those topics which have been successfully formulated in mathematical terms. . . . It is, perhaps, only if the biologist continues to do his best to cope with [mathematically difficult concepts] . . . with the imprecise but subtle tools of ordinary language that the mathematicians will eventually be driven to dissect them with their penetrating but relatively inflexible scalpels."

Professor Waddington writes with his characteristic lucidity and elegance, which is marred only rarely by a passage of 'purple' prose (see p. 88). There are many controversial points in the essays, for Waddington is an avowed neo-Lamarckian and tends away from the extreme atomism which is characteristic of much modern scientific thought. He has also dared to attack some sacred cows. Not every biologist will agree with all of Waddington's conclusions, but most will find these essays stimulating and thought-provoking; a very few will find them merely provoking.

S. J. COUNCE

5- HYDROXYTRYPTAMINE. G. P. Lewis, Ed. New York, Pergamon Press, 1958. 249 pp. $9.50.

This volume contains the papers presented at a Symposium organized by the British Biological Council's Co-ordinating Committee for Symposia on Drug Action, which was held in London on April 1 and 2, 1957. The contributors were obviously selected with care because, in the main, they

include a large number of the most productive and eminent workers in fields of research involving 5-hydroxytryptamine (5-HT, serotonin) and related biogenic amines. Possibly the most notable absentee, however, was Dr. V. Erspamer, the Italian scientist who is one of the co-discoverers of 5-HT and who has contributed importantly to the body of fundamental knowledge of this substance.

The book, like the Symposium, is conveniently subdivided into the following sections: Occurrence; Metabolism; Actions-cardiovascular; Actions-nerve endings and ganglia; Actions-carcinoid; Actions-anaphylaxis; Actions-gastric secretion; Peripheral Antagonists; Central Actions and Antagonists; and General Discussion. Of these, the least ambiguous and perhaps the least controversial information is to be found in the sections on occurrence, metabolism, and relation to the carcinoic syndrome. Everyone is generally agreed that 5-HT has a widespread occurrence in invertebrates, vertebrates, and in some plants; and, although this in itself may signify little, there is a uniform feeling that the substance is of some physiological importance. There is also good agreement on its formation from dietary tryptophan in a two-step sequence of reactions via 5-hydroxytryptophan. The oxidative deamination of 5-HT, catalyzed by monoamine oxidase, to form 5-hydroxyindoleacetic acid, is believed to be the principal route of biotransformation.

Except for Jacobson of Cambridge there is general acceptance that carcinoid tumors are neoplastic growths of the Kultshitsky (argentaffin) cells of the gastro-intestinal mucosa, which produce massive quantities of 5-HT. The elevated circulating levels of the amine can readily account for certain of the clinical signs observed: the diarrhea; the cutaneous vasomotor changes; the dyspnea on exertion. Jacobson believes that one should distinguish between argyrophil and argentaffin cells; and there has been some recent evidence which points to the pertinence of this argument.

When the rest of the picture of 5-HT is viewed broadly, i.e., the attempts to fit the many observed pharmacological effects of the amine into a scheme of physiological importance, the result is far from satisfactory or clear. The cardiovascular actions are such that Page has coined the term "amphibaric" to describe them. It is hardly possible to accept 5-HT as a causative agent in the genesis of any form of hypertension in view of its phasic action on blood pressure.

Mary Pickford of Edinburgh, from a series of elegant experiments conducted with her colleague, Abrahams, has concluded that the blood vessels of the dog's kidney show no particular sensitivity to the presence of 5-HT. In the rat, Erspamer had found an exquisite sensitivity of this vascular bed to 5-HT and the resulting antidiuresis prompted him to suggest that the major role of 5-HT is the control of renal function. We must now view this hypothesis as one relegated to the rat and closely related mammals.

Not even the function of 5-HT in platelets is clear. While it is tempting to ascribe to the amine in this site a role of vasoconstriction and hemostasis following agglutination. Stacy presents the argument that this may be far too simple a notion. Possibly the most fascinating finding of Stacy and his group has been the direct relationship between the level of 5-HT

and the level of ATP in platelets. The possibility exists that ATP may represent one of the anchoring sites for 5-HT in the body. At present, it seems most appropriate to look upon 5-HT in platelets as a transportation form of the amine, since in this locus, unlike most of the other sites in which 5-HT is found in high concentration, enzymes concerned with the formation of the amine are not present.

The role of 5-HT in anaphylaxis is complicated by several factors and, therefore, not well defined. Rodents contain large quantities of 5-HT in their mast cells and in their skins. Thus, in the rat, West has shown that 5-HT plays a major role in edema formation in the paw in response to the injection of foreign protein. The fact that 5-HT can cause a release of histamine, however, must be taken into account in such studies. Furthermore, it is recognized that histamine produces edema in the skin of the dog, cat, rabbit, and human, and that in these species 5-HT is much weaker in this respect. Finally, Herxheimer considers it doubtful that 5-HT causes any of the signs of anaphylaxis in the guinea pig, even though exposure of these animals to an aerosol spray of the amine results in a shock-like syndrome in a very short period of time. Here, as in other aspects of this problem, the ultimate role which 5-HT plays will have to await complete studies on the comparative pharmacology of the amine.

Possibly the most intriguing hypothesis of all is that 5-HT takes some part in neurophysiology. This is carried to an extreme by Woolley who feels that the amine is essential for normal mentation. Brodie and his colleagues have suggested that 5-HT is a transmitter of impulses in the central parasympathetic system which stands in functional antagonism to the central sympathetic system, mediated by norepinephrine. This theme has been arrived at largely through indirect evidence. In all fairness to these workers, however, it should be pointed out that they have used their working hypothesis well in allowing it to lead the way toward devising new experiments. Almost certainly, present views concerning the function of 5-HT in the nervous system constitute gross over-simplifications of incredibly complex situations; thus, they appear to ignore any role which acetylcholine may play in the central transmission of nerve impulses. There is, after all, no definitive evidence that 5-HT is a transmitter at all, in the sense that acetylcholine is at peripheral synapses.

On the whole, this is a useful compilation of current information on a substance of paramount interest. The fact that the symposium was held over a year ago does not, in this case, detract from the timeliness of the subject.

NICHOLAS J. GIARMAN

BIOCHEMISTRY OF SOME PEPTIDE AND STEROID ANTIBIOTICS. By E. P. Abraham. New York, John Wiley & Sons, Inc., 1957. ix, 96 pp. $3.00.

This little book presents the second series of CIBA Lectures in Microbial Biochemistry given in 1957 at the Institute of Microbiology, Rutgers, The State University of New Jersey. The author, one of the most productive investigators in the field of the chemistry of antibiotics, including the penicillins, discusses the developments to which he has been a distinguished contributor during recent years.

Chapter 1 is devoted to the Bacitracins, a remarkable group of polypeptides containing the thiazoline nucleus. With the exception of Lyman C. Craig of the Rockefeller Institute, no one has done as much as Abraham to advance our knowledge of the nature of these substances, the structure of which is now, except for a few details, completely established.

In Chapter 2 the author discusses the chemistry of the Cephalosporins, a chemically heterogeneous assembly of antibiotic substances elaborated by certain members of the *Cephalosporium* family. Cephalosporin P appears to be a steroid; Cephalosporin N is a penicillin containing a D-δ-aminoadipyl grouping in the position occupied by the phenylacetyl group in the classical benzylpenicillin; Cephalosporin C is chemically related to the N compound but yields no penicillamine in acid hydrolysis.

In Chapter 3 the relationships between structure and function of these and other antibiotics are discussed from the standpoint of modern biochemistry.

This stimulating and informative book, in which the text is generously supported by bibliographical references and index, is recommended to the attention of all organic chemists who are in any way interested in the rapidly expanding field of antibiotics.

HANS T. CLARKE

THE NEUROSES AND THEIR TREATMENT. By Edward Podolsky. New York, Philosophical Library, 1958. 555 pp. $10.00.

Dr. Podolsky in his foreword to this collection of papers writes that the volume came into existence to help "the physician in active practice" understand more about the diagnosis, management, and treatment of the neuroses.

In his attempt to do this, he has brought together 37 different papers written by specialists of varied caliber and belonging to markedly diversified schools of thought. Topics can be found to suit almost anybody's personal preference—to quote from the cover: ". . . from simple allergies to severe and crippling phobias—from psychotherapy in infancy to the handling of senility—from the routine psychotherapy to electroshock, from the new psychopharmacological aids to the extreme measures of lobotomy."

For a general practitioner this book would not be very helpful, one reason being that many of the articles are written for the specialist. Furthermore, the contradictory approaches and conflicting interpretations probably would only persuade the general practitioner that he should forget about the whole business.

For a psychiatrist there are many articles of great interest included in this collection: Spitz's excellent papers on infancy, Josselyn's work on children with rheumatic fever, Bick's study of occupational neuroses, and many others. This level of excellence, however, is not maintained; at times it dips markedly. One wonders about the need for a publication such as this. The book has no real unifying theme in spite of its title. Even as a reference book it is of questionable value as no specific references nor bibliographies are included.

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